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JUL 2 4 194

VOLUME 20 NUMBER 3

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CONTENTS

Boron in Citrus Trees. A. R. C. Haas, Citrus Experiment Station, Riverside, California 323

Effect of Mechanical Stimulation on the Electrical and Curvature Responses in the Avena Coleoptile. A. R. Schrank, University of Texas 344

Extent and Longevity of the Seminal Roots of Certain Grasses. J. E. Weaver and Ellen Zink, University of Nebraska 359

Effect of Light Intensity, Nitrogen Supply, and Fruiting on Carbohydrate Utilization by the Cotton Plant. Frank M. Eaton and Neil E. Rigler, Bureau of Plant Industry, Soils and Agricultural Engineering, College Station, Texas 380

Starch and Sugars of Vitis Vinifera. A. J. Winkler and W. O. Williams, University of California at Davis 412

Inducing Dormancy in Lettuce Seed with Coumarin. G. E. Nutile, Associated Seed Growers Inc., New Haven, Connecticut 433

A Simple and Effective Humidity Control. R. H. Wallace and R. J. Bushnell, University of Connecticut 443

The Respiration of Growing Plant Cells. Winifred Donnelly and W. A. Beck, Institutum Divi Thomae and Our Lady of Cincinnati College, Cincinnati, Ohio 448

(*Continued on Cover Page Four*)

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PLANT PHYSIOLOGY

JULY, 1945

BORON IN CITRUS TREES

A. R. C. Haas

(WITH EIGHT FIGURES)

Introduction

During the past twenty-five years attention has been given to the effects of boron on trees. Practically all soils in southern California were then considered to be alkaline in reaction and under such conditions boron availability might be interfered with. Recently, however, it was found that soils in healthy citrus orchards are generally acid (**11**) at some time during the year; some soils are becoming so acid as to require calcium carbonate. Also recently (**10**) it was shown that most citrus soils in southern California contain adequate amounts of available boron.

Boron is an essential element for citrus (**8**). The response of olive trees to applications of boron to the soil (**25**) has afforded an opportunity for studying adjoining citrus trees in low-boron areas. Excessive-boron areas on the other hand are numerous. Use was made of trees growing in both of these and in healthy areas in supplementing studies of trees growing under controlled conditions in artificial cultures.

In Rhodesia (**18**) a condition known as "hard fruit" in navel orange trees was corrected by applications of boron to the soil. Translucent spots, such as were found in artificial cultures (**13**), were noted and in addition there were deposits of gum around the fruit axis and in the albedo of the rind. No corky split veins were observed in the leaves. In Florida (**22**) grapefruit trees were grown in (coarse builders' sand) cultures and when the trees were boron-deficient, the fruit showed brown discolorations in the albedo but no gum was noticed around the axis of the fruit such as was described in Rhodesia. In Florida but not in Rhodesia there was observed a thickening of the midrib and veins, the midrib of the old leaves becoming corky (**1**) on the dorsal surface. Other observations (**3, 4**) in Florida groves revealed symptoms similar to those reported from Rhodesia but the symptoms were not consistent over a period of time in the same grove, disappearing rather quickly when a resumption of rains ended a period of unusual drought. This view is consistent with the results obtained on the avail-

ability of boron in soils in which it was found (10) that as the dilution of the soil increases the water-soluble boron (air-dry soil basis) increases.

The symptoms of deficiency and of initial stages of excess boron in citrus are not well understood. These symptoms together with the solubility or availability and the accumulation of boron in citrus grown under various conditions are herein presented.

Methods

The turmeric method (10) was used for boron tests in citrus. Various amounts of boron (boric acid) were added to ground leaf powder dried at 65° C. This powder is hygroscopic. Recovery values (table I) were obtained which are quite satisfactory.

TABLE I

RECOVERY OF BORON WHEN ADDED TO VARIOUS AMOUNTS OF GROUND LEMON LEAVES FROM CUTTINGS GROWN IN LOW-BORON CULTURE SOLUTIONS

SAMPLE OF DRY MATTER (BORON-DEFICIENT LEMON LEAVES)	BORON ADDED	TOTAL BORON FOUND	RECOVERY OF ADDED BORON	
gm.	*mg.*	*mg.*	*mg.*	*%*
0.2	0.000	0.004,7	.....	.....
0.2	0.010	0.015,2	0.010,5	105
0.2	0.020	0.025,7	0.021,0	105
0.4	0.000	0.009,4	.....	.....
0.4	0.010	0.018,6	0.009,2	92
0.4	0.020	0.028,3	0.018,9	95
0.6	0.000	0.015,1	.....	.....
0.6	0.010	0.025,3	0.010,2	102
0.6	0.020	0.033,0	0.017,9	90
0.8	0.000	0.017,0	.....	.....
0.8	0.010	0.026,3	0.009,3	93
0.8	0.020	0.037,0	0.020,0	100
1.0	0.000	0.021,0	.....	.....
1.0	0.010	0.031,7	0.010,7	107
1.0	0.020	0.040,7	0.019,7	99
0 + 1 gm., no. 42, 9 cm. diam., Whatman filter paper	0.010	0.010,1	.....	101

Leaves and fruits were washed in running distilled water and wiped dry. After being weighed, they were dried at 65° C., ground in a Wiley mill, and redried. For water-soluble boron a suspension of 1 to 2 grams of dry material was made in 50 to 75 ml. distilled water. After several hours the suspension was filtered with the aid of suction through a platinum, cone-supported filter paper in a funnel inserted in the stopper in the top of a bell jar with suction at the side outlet. The filtrate together with 10 ml. of calcium hydroxide solution (1.85 gm. in 500 ml.) was evaporated to dryness in a Vitreosil dish. The residue and filter paper were likewise dried. Each was heated over a Fisher burner with air vents closed. The cooled ash was leached three times with distilled water and the rinsed filter paper was dried and reignited in the original dish until the ash was white. After very slight

acidification with dilute HCl, heating of the solution on the hot plate and filtering through the original funnel, the volume was brought near 100 ml. and completed when cool. Usually 5- and not over 25-ml. aliquots were pipetted in duplicate to porcelain dishes as described elsewhere (10) for completing the colorimetric determination of the boron. Duplicate tests of total boron were also made and their agreement with the added values of both boron fractions was satisfactory.

Results and discussion

Symptoms

For the study of boron deficiency in citrus fruits it was necessary to grow the trees first in culture solutions containing boron (0.1 p.p.m.) until the fruits were partially grown and then at various stages of fruit growth

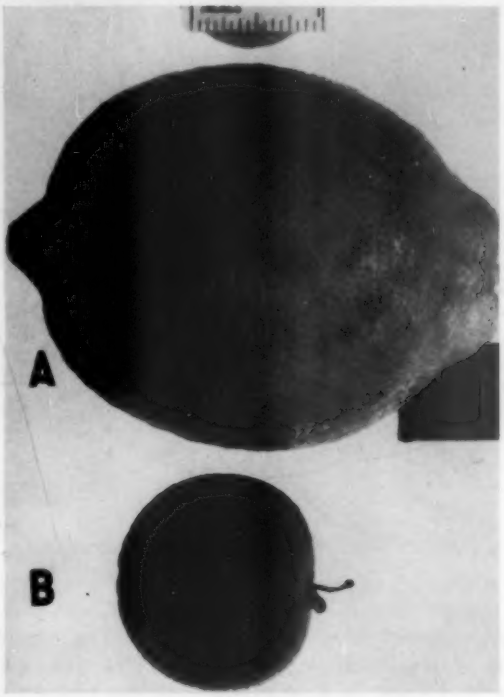

FIG. 1. A, mature lemon fruit; B, immature navel orange fruit. Trees grown in culture solutions which initially contained 0.1 p.p.m. boron and then no boron. In A, dark-colored, gum-soaked areas occur in the peel; gum exudations are marked by black lines. In B, the exudation is near the retained style of the pistil (navel or blossom end).

to omit the boron. Otherwise, flowering was scant and of the fruits that set there was an excessive premature abscission, gum exudations being frequently seen at the tip of flower pedicels after the abscission of the flowers. Some of the fruits from boron-deficient trees contained gum in the albedo of the rind although the number of fruits without such gum was by far the greater.

In figure 1 are shown some external effects of boron deficiency in lemon and orange fruits. In severe cases under glasshouse conditions, gum may be forced through the peel (lemons) or accumulate about the core (navel oranges) until the gum protrudes through at the blossom end. Figure 2 shows such affected fruits when cut transversely at the middle diameter. Gum is indicated in L as occurring in the albedo of the lemon rind. A seed is shown without gum in one of the lemon fruit segments.

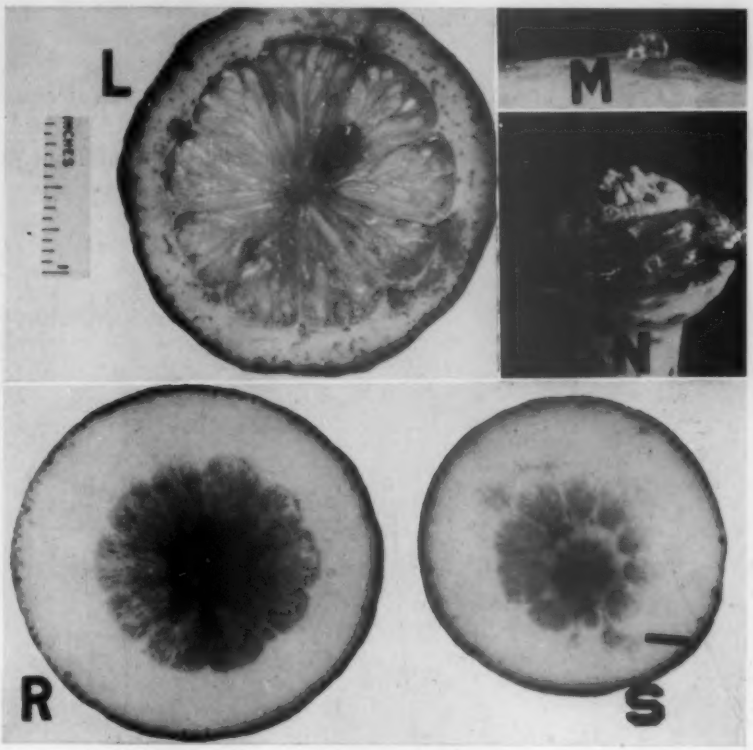

FIG. 2. Boron deficiency effects in lemon and navel orange fruits obtained from trees grown in solution cultures: L, a lemon fruit cut open to show the gum in the rind and to show the healthy-appearing seed; N, fruit stalk with gum exuding from the newly exposed surface after the detachment of the fruit and an enlargement (M) of such a gum deposit; R, a young navel orange fruit cut open to show that the gum occurs in the center (core) of the fruit and none in the angles of the fruit segments; S, same as R, but in addition also has gum occurring in the albedo of the rind.

When fruits of boron-deficient trees are pulled or fall from the fruit stalk or pedicel, there frequently appear small exudations of gum (one of which is enlarged in M) on the newly exposed surface and severed portion of the stalk. This is in agreement with observations made in Florida (**22**).

Pockets of gum were sometimes found in the core or along the secondary fruit stalk and in the albedo of the rind of young navel orange fruits (R and S, fig. 2). In Florida (**22**) no gum was found around the axis of the fruit.

The location of the gum in boron deficiency is quite different from that in exanthema (copper deficiency): in the former, gum may occur within the albedo of the rind and in the central core; in the latter, the gum occurs in the outside (flavedo) portion of the rind and within the angles of the segments.

Boron-deficient trees may show splits (fig. 3) in the bark of branches, trunk or rootstock. The vessels in cross section are often filled with gum and when pruning is done, exudations of gum may appear at the cut surface. Even without pruning the tension resulting from gum accumulation may force the gum through the bark.

Gum formation results in marked growth disturbances in the leaves of boron-deficient trees. Healthy leaves (fig. 4) have long narrow palisade cells below the dorsal epidermis while in boron-deficient leaves such cells are round and are separated by gum.

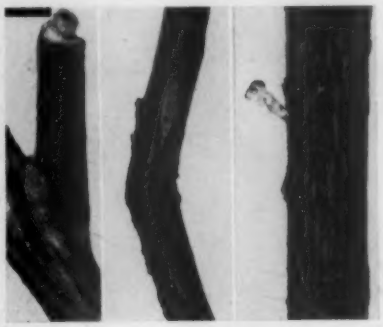

FIG. 3. Navel orange twigs from trees in solution cultures lacking boron: left, gum exudation on the cut surface; middle, a split in the bark; and right, gum protruding through the bark of a branch.

Boron-deficient leaves generally are brittle and may curl. This, together with the corky split veins may be largely the result of a high osmotic pressure brought about by the accumulation of sugars (**12**) which in turn is caused by interruptions in the conducting tissue.

On the left in figure 5 is shown an initial stage in the corking and splitting of the veins of boron-deficient lemon leaves. Translucent spots become numerous and the veins become conspicuous. In the middle (fig. 5) is shown the pitting or indentations that may occur in the lower surface of the leaf (**17**, p. 150), but which were found in only a few cultures. Some citrus varieties, however, are considered as having such pitting as a normal condition in healthy trees. To the right in figure 5 are shown some external effects of internal gum as viewed from the lower surface. Accompanying such gum deposits, between the cells in the affected leaves, is the spotting and distortion of the leaves as seen in figure 6. This shoot was obtained from a lemon tree grown in a culture solution in which 0.1 p.p.m. of boron was used initially, during which time fifteen large fruits were produced after which boron was omitted.

In figure 7 is a portion of two cycles of growth in which every leaf was dark green at first and had affected veins. These leaves became brittle and yellowish-green before abscission as the tip of the shoot died back. It was found that the presence of fruits delayed the initiation of such leaf symptoms and indicates that boron is not fixed in an immobile form (**6, 22**).

The initial stages of excessive boron in citrus leaves (**7**) are sometimes

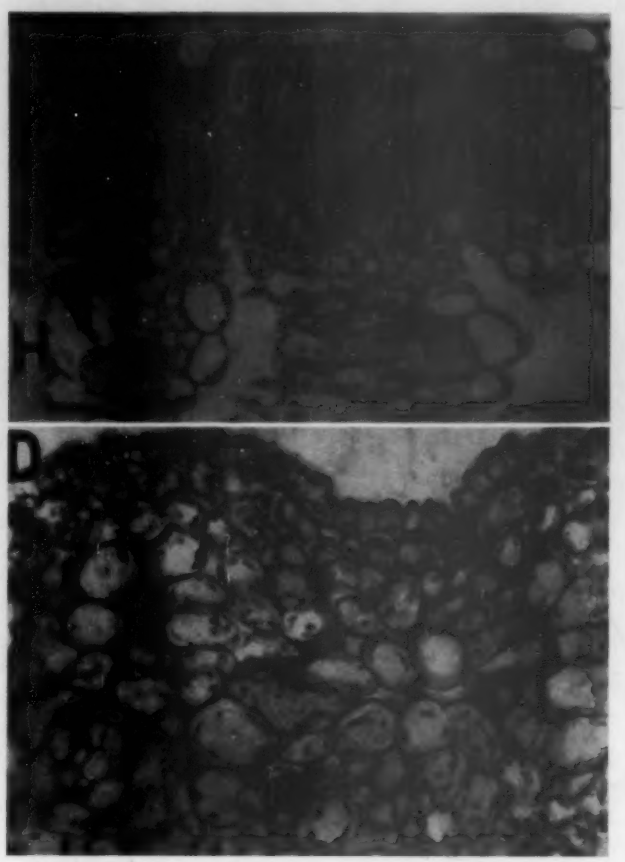

FIG. 4. The presence of gum and of rounded instead of narrow elongated palisade cells in transverse sections of boron-deficient lemon leaves: H, section of a leaf from a healthy tree grown in a solution culture with a maintained boron concentration of 0.1 p.p.m.; D, section of a leaf from a tree grown in a solution culture similar to H, but in which the boron after a time was omitted.

difficult to diagnose. Figure 8 shows the slight yellowing of the leaf tips followed by a slight tip burn, the main portion of the blade remaining dark green. The slight tip burn might be mistaken for initial stages in excessive chlorine, urea, high sulfate, and other factors (**9**, p. 497; **14**). Nitrate of soda was suspected of containing excessive boron when used on soil in a lemon orchard near Elsinore in which the leaves became yellow at the tip.

It was found, however, that the fertilizer was not faulty and that the leaves contained a normal total boron content (64.75 p.p.m. in the dry matter). In a similar case at Rialto, Valencia orange leaves were found to have a total boron content of 43.51 p.p.m.

It is the desire of most investigators that deficiencies or excesses be subject to diagnosis from one or more specific symptoms which are characteristic solely of the given element or factor. In practice this seldom happens and it is found that in utilizing growth reactions as a means of diagnosis that

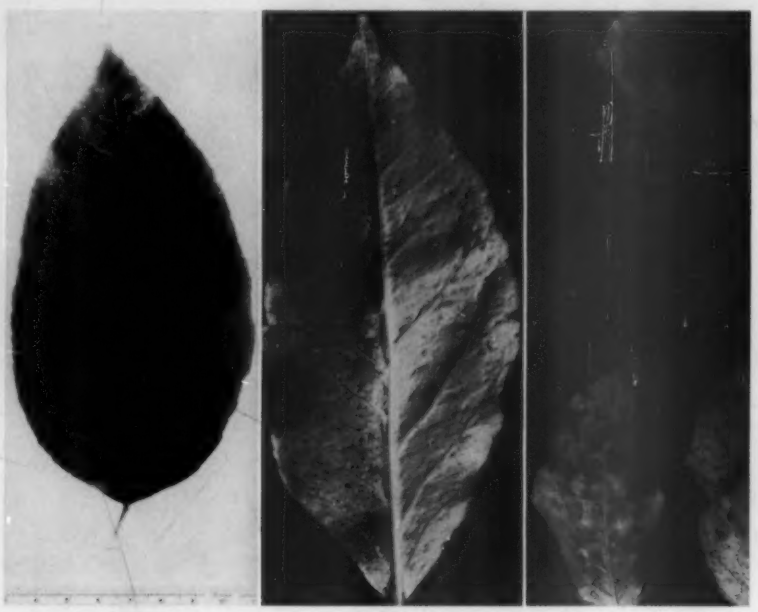

FIG. 5. Boron-deficiency effects in lemon leaves from trees grown in culture solutions: left, translucent spots that may precede the splitting and corking of veins in the upper surface; middle, indentations or pitting in lower surface; right, external effects of internal gum as seen on lower surface.

it is essential that all of the available symptoms be considered as a whole; this includes the symptoms (deficiency and excess) of the elements thus far studied. Symptoms and chemical analyses serve best when they are considered more as supplements to each other and as useful guides rather than as finalities in themselves.

LOW BORON IN CITRUS ORCHARDS

In the Oroville area olive trees have shown marked response to soil applications of boron (**25**) although adjoining unthrifty navel orange trees have not as yet shown any response. The boron contents of citrus leaves and fruits are shown in table II. The application of borax to the soil increased the boron content of the leaves. In the June 25th samples the water-insoluble boron remained unchanged. One-half pound of borax increased the

boron content of the peel in ripe fruit while in the pulp the total boron content increased only slightly. Immature whole fruits showed small increases in boron content when a soil application of borax was made. From numerous boron tests on leaf samples elsewhere it certainly appears from the analyses that the leaves of untreated trees (table II) are very low or deficient in boron. No corky split veins or gum in the fruit rind or any other symp-

FIG. 6. Boron-deficient, immature lemon leaves from near the tip of a growing shoot. Lower surface of leaves appears as shown to the right in figure 5. The internal gum causes distortion and spotting.

toms were evident in the samples used in table II, although the trees are reportedly in an unthrifty condition (**25**). Soil tests for available boron also showed a low boron availability (**10**) in the soils represented in table II. Tests on soils of some other citrus orchards in the same county showed adequate boron availability (**10**).

Although the December 28th samples of leaves (table II) differ markedly in their boron content, their total Ca and Mg in the dry matter show no ap-

preciable differences: 5.18 and 0.54 per cent., respectively, for the control; and 5.03 and 0.50 per cent., respectively, for the treated. Tissues of plants inadequately supplied with boron were reported in other plants to contain

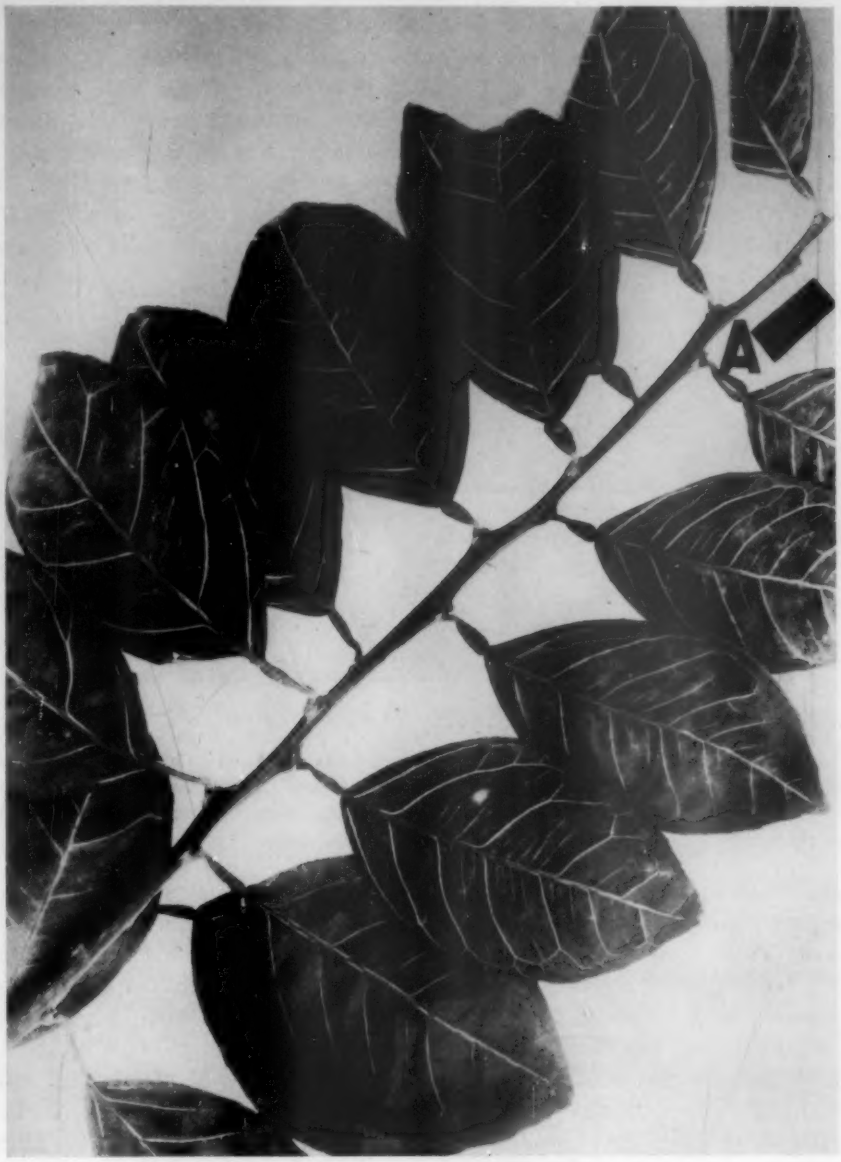

FIG. 7. Boron-deficient navel orange leaves on one of two successive cycles of twig growth in which every leaf had corky split veins on their upper surface and was very brittle. The boron-deficient culture solution initially contained 0.1 p.p.m. boron and then no boron. The addition then of 0.1 p.p.m. boron brought about new growth without such vein injury. Length of A is one inch.

higher percentages of Ca and Mg than those having an adequate supply (**6, 15, 19**).

In healthy orchards and in solution cultures occasionally a twig is found bearing a few leaves with corky split veins without boron being involved. For example leaves of a few trees in a small lemon orchard showed such effects. These few trees were heavily infested with both mites and scale, and growth was severely checked whereas in adjoining non-infested trees no vein effects were observed. The affected leaves contained 55.71 p.p.m. water-soluble boron and 15.74 p.p.m. water-insoluble boron in the dry matter while the available boron in the soil was 1.21, 1.01, and 0.58 p.p.m., respectively, in the top three feet. When leaves are severely nitrogen starved or when leaf cuttings are rooted and unable to continue top growth, corky split veins may

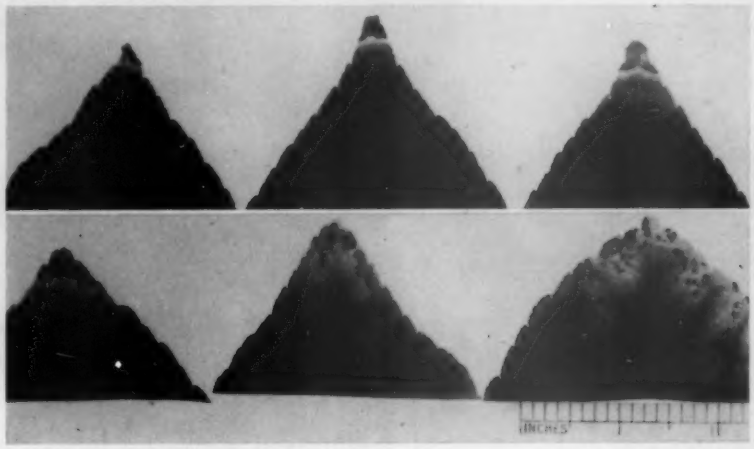

FIG. 8. Initial stages of boron excess in lemon leaves from trees grown in large sand cultures receiving low concentrations of potassium: top row, upper surface of leaves showing a slight yellowing of the tip followed by a slight tip burn; bottom row, lower surface of leaves showing in addition some small scattered gum spots.

make their appearance without boron being a factor. Large accumulations of carbohydrates may be a partial explanation for the vein effects in such cases (**12**). In a large healthy navel orange orchard in Riverside, a single twig bearing a few leaves with corky split veins was found. The dry matter of these leaves contained 143.75 p.p.m. water-soluble and 24.90 p.p.m. water-insoluble boron. In excellent navel orange orchards near Highgrove and at Bryn Mawr a few trees were found bearing symptoms of magnesium deficiency in the leaves, some of which showed corky split veins. In one case the water-soluble and water-insoluble boron values in the dry matter were 61.20 and 17.50 p.p.m. and in the other case 274.07 and 27.72 p.p.m., respectively. In a magnesium-deficient solution culture in the glasshouse the leaves of an orange tree recently showed corky split veins when boron was not deficient. These results with magnesium-deficiency are in harmony with the observations made in magnesium-deficient orchards in Florida (**3**). Corky split

TABLE II

BORON CONTENT OF NAVEL ORANGE LEAVES AND FRUITS FROM TREES IN A LOW-BORON SOIL AREA NEAR OROVILLE, CALIFORNIA*

PORTION OF TREE	DATE OF COLLECTION	CONDITION OF MATERIAL	SOIL TREATMENT	BORON IN DRY MATTER	
				WATER-SOLUBLE	WATER-INSOLUBLE
	1943			*p.p.m.*	*p.p.m.*
Leaves	Dec. 28	Mature	Control	3.78	13.02
Leaves	"	"	0.5 lb. of borax applied Dec. 10, 1942	133.75	17.63
Leaves	Sept. 30	"	.....	1.14	8.78
Leaves	June 25	"	Control	9.40	12.50
Leaves	"	"	0.25 lb. of borax applied Feb. 19, 1943	76.73	12.79
Peel	Dec. 22	Ripe fruit	Control	4.24	8.92
Peel	"	" "	0.5 lb. of borax applied Dec. 10, 1942	11.03	13.98
Pulp (navel discarded)	Dec. 22	" "	Control	4.41	9.35
Pulp (navel discarded)	"	" "	0.5 lb. of borax applied Dec. 10, 1942	10.31	5.75
Whole fruits	June 25	Immature (2 cm. × 2.4 cm.)	Control	11.57	10.28
Whole fruits	"	Immature (2.3 cm. × 2.4 cm.)	0.25 lb. of borax applied Feb. 19, 1943	14.21	12.73
Peel	Sept. 30	Green, immature fruit	.....	3.54	8.50
Pulp (navel discarded)	"	" "	.....	2.04	4.46

* Material obtained through the kindness of MR. H. P. EVERETT, County Agent, and MR. C. E. SCOTT, Extension Specialist in Plant Pathology.

veins have been reported (**5, 20**) for low and excessive potassium cultures. Affected leaves thus far have not been observed in the leaves of our low potassium cultures, although splits in the bark of the twigs have been found.

LOW BORON IN GLASSHOUSE CULTURES

Table III gives the boron content of trees grown in culture solutions. When leaves with corky split veins were analyzed, the boron content in both fractions was very low, especially the water-soluble fraction. With slightly increased soluble boron content (2.46 or 3.23 p.p.m.) the veins were free of corky splits and the insoluble boron in both cases increased to about 10 p.p.m.

The first two leaf samples in table III were tested for the solubility of Ca, Mg, and K. Boron-deficient leaves (without corky split veins) showed (as percentage of dry matter) water-soluble: Ca, 1.68; Mg, 0.36; K, 1.40; water-insoluble: Ca, 2.21; Mg, 0.08; K, 0.06. Boron-deficient leaves (with corky split veins) in the same cultures showed: water-soluble, Ca, 1.18; Mg, 0.30; K, 1.48; water-insoluble, Ca, 1.95; Mg, 0.10; K, 0.09. Without affected veins 43.08 per cent. of the Ca, 81.59 per cent. of the Mg, and 95.63 per cent. of the

K were water-soluble, whereas with affected veins 37.64 per cent. of the Ca, 75.45 per cent. of the Mg, and 94.15 per cent. of the K were water-soluble. In boron-deficient leaves, therefore, in table III, the leaves without the corky

TABLE III

BORON CONTENT OF CITRUS LEAVES AND BARK OF TREES GROWN IN BORON-DEFICIENT CULTURE SOLUTIONS IN THE GLASSHOUSE

VARIETY	SAMPLE	DESCRIPTION OF SAMPLE	BORON IN DRY MATTER	
			WATER-SOLUBLE	WATER-INSOLUBLE
			p.p.m.	*p.p.m.*
Navel orange on sweet orange stock	Leaves	Curled; brittle; veins corky and split	0.31	5.43
	Leaves	Curled; brittle; veins not affected	2.46	10.08
	Green twigs	Up to one-fourth inch in diam.	0.65	4.50
	Trunk bark	Gum in wood	2.00	10.20
	Rootstock bark	Gum in wood	1.10	15.71
Navel orange on sweet orange stock*	Leaves	Veins corky and badly split	1.11	6.45
	Leaves	Veins not corky or split	3.23	9.70
	Green twigs	Up to one-half inch in diam.; gum protruding through bark; corky splits in twigs	4.82	13.79
Healthy Eureka lemon on sweet orange stock	Leaves	Healthy; 0.05 p.p.m. boron in culture solution	24.13	9.63
	Trunk bark	Above bud union	20.50	26.75
	Rootstock bark	Below bud union; above first root	7.50	21.88
	Root bark†	Primary root bark	20.86	15.48
	Root bark	Secondary root bark	23.63	12.13
Lemon cuttings	Leaves	Veins badly split; no boron in culture solution	3.05	6.15
	Leaves	No vein injury; 0.005 p.p.m. boron in culture solution	11.15	14.90
	Leaves	No vein injury; 0.1 p.p.m. boron in culture solution	29.70	16.38
	Leaves	Same as above; sample 4 months earlier	36.10	18.75
	Bark	From branches and trunk; gum under bark	5.62	21.50
Lemon cuttings (control)	Leaves	Healthy; 0.25 p.p.m. boron in culture solution	130.15	15.74
Valencia orange cuttings (control)	Leaves	Same as above	99.15	16.90

* Leaves of that portion of the tree bearing 2 large fruits, are healthy in appearance.
† Citrus root bark of orchard trees at Riverside contained 13.59 p.p.m. water-soluble boron and 18.60 p.p.m. water-insoluble boron in the dry matter.

split veins contained more soluble Ca and Mg than did the leaves with affected veins and is in agreement with the results of other investigations (**2, 16, 23**).

The boron content of fruits from boron-deficient trees grown in solution cultures is reported in table IV. In navel orange fruits the soluble boron

content was greatly reduced in both the peel and pulp. The variable boron content is probably due to first having grown the trees with boron. Some trees accumulated more boron than others and the amount of growth varied. With the reduction of boron in the peel only a few fruits showed gum formation. The boron content of a fruit is probably related to the number of

TABLE IV

BORON CONTENT OF MATURE CITRUS FRUITS FROM TREES GROWN IN BORON-DEFICIENT CULTURE SOLUTION

VARIETY	TREE SYMPTOMS	BORON IN DRY MATTER OF FRUITS			
		PEEL		PULP	
		WATER-SOLUBLE	WATER-INSOLUBLE	WATER-SOLUBLE	WATER-INSOLUBLE
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
	Corky split veins	20.08	10.32	11.13	4.00
	Corky split veins; no gum in fruit; peel thin; granulation	20.45	6.70	10.95	2.83
	Corky split veins; no gum in fruit	6.49	5.91	2.57	2.94
Navel orange on sweet orange stock	Granulation; no gum in fruit	3.19	6.50	3.75	2.70
	None	12.30	12.40	6.16	6.40
	Peel thin; gum near core or stalk in center of pulp	1.73	2.63	0.70	1.94
	Same as above	1.51	3.58	0.90	1.74
	No gum in fruit; gum in twigs; corky split veins; roots poor	5.71	15.10	7.86	6.34
	Gum in trunk; top died when boron was supplied; rootstock recovered	3.35	4.85	3.55	2.80
	Healthy (control)	21.44	16.50	10.84	10.18
Valencia orange cuttings	Peel thin; no gum in fruit	9.71	14.21	.....	.....
	Gum in stem end of peel	7.65	16.70	8.59	3.83
	Healthy (control)	12.88	13.83	12.00	5.76
	" "	14.38	18.85	.....	.....
Lemon cuttings	Leaf injury; no gum in fruit	9.71	5.76	3.46	4.63
	Fruit silver without gum	5.57	9.25	2.93	3.69
	New growth killed; brown in peel	9.74	8.91	1.31	4.54
	Healthy (control)	11.54	11.87	10.00	3.46

leaves per fruit. The presence of numerous fruits appreciably retarded the deficiency effects in the leaves.

The lemons on a boron-deficient cutting grown in solution culture were full picking size and green except where noted. In their peel the water-soluble and -insoluble boron contents (p.p.m. in dry matter) were, respectively, 4.81, 4.58; 3.02, 4.31; 4.99, 6.59; 6.38, 6.27 (ripe); 4.65, 6.39; 2.03, 8.81; 3.62, 6.28; 4.45, 7.66; 9.33, 4.53.

BORON IN CITRUS FRUIT JUICE

The juice of hand-reamed fruits was strained free of most of the pulp and was centrifuged to 840 r.p.m. for 15 minutes and filtered with suction.

Duplicate 25-ml. aliquots to which 10 ml. of calcium hydroxide solution was added, were evaporated to dryness, ashed, and brought to a volume of 100 ml. as described under "Methods."

As shown in table V the boron content of navel orange juice differed in fruit samples collected the same day in widely separated locations. The juice in the tip half of pulp contained more boron than that in the stem half. Grape-fruit juice contained very much smaller concentrations of boron than navel orange juice.

Maturity of fruit and boron content

Samples of Valencia orange fruit (15 to 20 fruits per sample) were collected at various times from the same trees in the Plant Pathology plots at

TABLE V

Boron content of citrus fruit juice

Date	Location of orchard	Variety	Portion of pulp juiced	Boron in juice
				p.p.m.
Jan. 28, 1943	Indio	Navel orange	All	1.36
" "	Mentone	" "	"	1.43
" "	Citrus Experiment Station, Riverside	" "	"	1.59
Feb. 25, 1944	Citrus Experiment Station, Riverside	" "	Stem half	1.16
		" "	Tip "	1.27
Mar. 6, 1944	Citrus Experiment Station, Riverside	" "	All	1.31
Feb. 16, 1944	"Acorn-diseased" trees, Redlands	" "	Stem half	0.93
		" "	Tip "	1.25
		" "	All	0.99
Mar. 25, 1943	Thermal	Marsh grapefruit	All	0.40
Mar. 28, 1943	Thermal	" "	All	0.50

the Citrus Experiment Station. The buttons were removed and the fruits were lightly brushed in running distilled water after which they were wiped dry and weighed and the peel separated from the pulp. The peel was then dried at 65° C. in a ventilated oven and ground in a Wiley mill.

Table VI gives the water-soluble and water-insoluble boron content in the peel of Valencia orange fruits at various times after the peel was fully colored. Except in the final sampling, there was more water-soluble than insoluble boron in the dried peel. The total boron content of the peel increased after the fruits became fully colored.

Eureka lemon fruits[1] of various sizes (1.5 to 4.5 cm. in diameter) were collected from an orchard at Sespe and the fruits (ranging from 8 to 44 per sample) were dried without separation of peel and pulp. No trends could be seen in the values for the boron fractions. Likewise at Riverside, Eureka lemon fruits of various sizes (0.5 to 5.5 cm. in diameter) were collected and the fruits (ranging from 7 to 336 per sample) were dried and analyzed and

[1] Collected through the kindness of Dr. F. F. Halma.

TABLE VI

EFFECT OF AGE OF VALENCIA ORANGE FRUITS ON THE BORON CONTENT OF THE DRIED PEEL

DATE OF COLLECTION	NO. OF FRUITS	BORON CONTENT IN DRY MATTER OF PEEL		
		WATER-SOLUBLE	WATER-INSOLUBLE	TOTAL
1942		*p.p.m.*	*p.p.m.*	*p.p.m.*
Mar. 14	12	16.09	13.46	29.55
April 8	20	15.09	14.57	29.66
May 9	18	21.19	11.70	32.89
June 10	16	20.40	13.87	34.27
July 11	16	20.86	12.86	33.72
Sept. 11	15	17.81	25.57	43.38

TABLE VII

BORON CONTENT IN PEEL AND PULP OF LEMONS AND GRAPEFRUIT OBTAINED IN THE ORCHARD

ORCHARD	DATE OF COLLECTION	NO. OF FRUITS	CONDITION OF FRUITS	BORON IN DRY MATTER			
				PEEL		PULP	
				WATER-SOLUBLE	WATER-INSOLUBLE	WATER-SOLUBLE	WATER-INSOLUBLE
			LEMON				
				p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Rubidoux plot F (manure)	Feb. 25, 1930	15	Ripe	13.68	11.70	.....	.....
Rubidoux plot B (check)	" "	15	"	6.70	17.48	.....	.....
Oxnard (high-boron area)	June 9, 1942	5	Green	17.08	17.40	18.80	6.17
		5	Tree ripe	16.02	17.67	20.85	6.72
Carlsbard	June 16 1941	13	Green	18.95	14.63	6.15	7.80
		8	Silver or tree ripe	12.25	13.18	5.94	5.71
Fallbrook	Dec. 7, 1938	.....	Ripe	7.53	14.96	5.69	10.40
Hueneme	Dec. 2, 1938	23	"	12.91	12.87	8.30	10.59
Oxnard	" "	21	"	13.05	15.30	8.75	12.15
			GRAPEFRUIT				
Thermal	Mar. 25, 1943	.....	Marsh	18.23	18.15	6.92	2.77
Mesa, Ariz.	Mar. 12, 1943	3	Marsh*	17.02	15.10	9.40	2.93
C. E. S. Riverside Field 12, R 8, T 1, 2	Mar. 12, 1943	3	Marsh†	4.41	15.20	7.68	3.31

* Hard gritty brown areas in albedo beneath surface humps.
† Healthy, not fully mature fruits.

again the results showed no trend. Future tests must take into consideration the age rather than size, the scion and stock variety, and other factors.

Boron in citrus leaves and fruits in orchards

Samples of lemons and grapefruits were collected in which the peel and pulp were separated and their boron solubility determined. The pulp of each fruit was halved so as not to lose any juice. After drying at 65° C. the halves were broken up and further dried in the glass dishes and then were pulverized in a mortar.

The results in table VII show that ordinarily the peel contains a higher concentration of boron than the pulp even though the seeds were included in the pulp. In fruits from the Rubidoux plots, although the total boron was the same, the peel of lemons from the check plot contained less soluble boron than that of the fruits of the fertilized plot. In the high-boron area there was a notable increase in the water-soluble boron in the pulp of lemon fruits.

The grapefruit samples showed uniform boron concentrations in any given fraction except in the water-soluble fraction of immature fruits. The sample from Mesa is of interest because of the resemblance in the peel to that of boron-deficient grapefruit. Analysis showed that the fruit was not deficient in boron even though those who first received it at once tentatively diagnosed the trouble as being a deficiency of boron. No letter accompanied the fruit, hence the orchard could not be located.

Borax was applied (broadcast) to the soil (Hanford sandy loam) in the tree square in a navel orange orchard in West Ontario on April 8, 1940.[2] A uniform row of trees was first selected. The check tree was near the top of the irrigation row and the borax treatments increased progressively down the row. On January 26, 1942, samples of fruits and leaves were collected. The water-insoluble boron fraction in the leaves was uniform (table VIII) regardless of the fact that the water-soluble fraction in the leaves increased in a manner coresponding to the amount of borax applied. The leaves at the higher borax applications resembled those shown in figure 8. The data (table VIII) show no relation of the boron applied to the soil and that found in the peel, although the boron in the pulp appears to show a relation to the borax applied (**18**, **24**). The secondary fruit or navel portion in the fruits was discarded because of the difficulty of separating peel and pulp. This may be a factor to consider.

No evidence was found in these leaves to show that soluble boron increases the soluble Ca or Mg (**16**). With increasing boron in the leaves, the values for the water-soluble Ca as percentage of total Ca in the dry matter were: 41.08 (check), 39.86, 38.39, 38.90 and 38.27. Similarly, the Mg solubility values were: 85.56 (check), 87.19, 83.84, 88.80 and 86.12, respectively.

When boron was added to the irrigation water in a portion of a uniform

[2] Assistance was kindly given by Mr. R. G. LaRue, then Assistant Farm Advisor in San Bernardino County.

acreage of lemon trees, the leaves showed an increased boron content. With boron added to the irrigation water, the water-soluble boron in the dry matter of the leaves was 63.61 p.p.m. and the water-insoluble boron 16.85 p.p.m., whereas the values in the check leaves were 21.62 and 15.00 p.p.m., respectively.

In table VIII the total boron in Valencia orange peel, pulp, and leaves exceeded the values found with the highest borax treatment with navel orange trees.

TABLE VIII

BORON IN CITRUS FRUITS AND LEAVES AS AFFECTED BY THE BORON CONTENT OF THE SOIL

ORCHARD AND DATE OF COLLECTION	BORAX ADDED TO SOIL OF TREE SQUARE	BORON IN DRY MATTER					
		PEEL		PULP		LEAVES	
		WATER-SOLUBLE	WATER-INSOLUBLE	WATER-SOLUBLE	WATER-INSOLUBLE	WATER-SOLUBLE	WATER-INSOLUBLE
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Navel orange at W. Ontario Jan. 26, 1942	Check (none)	13.19	12.57	13.94	7.85	103.39	20.62
	8 ounces	11.65	14.93	16.88	7.29	169.69	19.92
	12 "	15.52	10.16	16.15	10.38	264.65	18.53
	16 "	15.78	8.75	17.50	8.88	325.00	19.35
	20 "	13.34	12.15	20.05	7.65	440.00	20.72
Valencia orange at Oxnard, June 19, 1942	None; high-boron area	17.23	15.70	23.50	9.05	478.5	38.35
Lemon at Oxnard, June 19, 1942	" "	.....	.....	.....	.....	618.9	34.12
Lemon at Costa Mesa	" "	.....	.....	.....	.....	403.3	27.80

BORON SOLUBILITY

As shown in table IX the use of 0.1 N HCl as extractant reduced the insoluble boron content far below that brought about by distilled water (**27**). Some citrus plant tissues are quite acid and more boron than is indicated by the water-soluble fraction may thereby become available.

Large additions of calcium carbonate in conjunction with calcium nitrate (table X) resulted in a lower water-soluble boron content in the dry matter of Valencia orange leaves than when sulphur and ammonium sulphate were used (**26**). In the dry matter of navel orange leaves from nitrogen-starved trees (check plots), the water-soluble boron was notably increased above the concentration found in leaves from trees in nitrogen plots. When potassium is deficient instead of nitrogen, the leaves also accumulate boron as reported for lemon plants (**5**). In two series of large sand cultures with both lemon and orange cuttings grown with 0.1 p.p.m. boron in the culture solution and various concentrations of potassium, it was found that in the low potassium range the leaves showed symptoms of boron excess (fig. 8). In the dry

TABLE IX

EFFECT OF ACID EXTRACTION AT ROOM TEMPERATURE ON THE INSOLUBLE BORON CONTENT OF THE DRY MATTER OF CITRUS LEAVES AND PEEL

DATE OF COLLECTION	SOURCE OF MATERIAL	INSOLUBLE BORON IN DRY MATTER	
		WATER AS EXTRACTANT	0.1 N HCl AS EXTRACTANT
		p.p.m.	*p.p.m.*
March 25, 1943	Marsh grapefruit peel; Thermal	18.15	0.80
June 26, 1943	Navel orange leaves (sweet orange stock); corky split veins; boron-deficient solution culture	6.45	1.65
April 5, 1943	Marsh grapefruit leaves; Citrus Experiment Station Pathology Plot, Riverside	13.98	2.65
" "	Eureka lemon leaves; Citrus Experiment Station Pathology Plot, Riverside	11.05	3.80
" "	Navel orange leaves; Citrus Experiment Station Pathology Plot, Riverside	14.62	3.65
June 20, 1942	Valencia orange leaves; high-boron area; Oxnard	38.35	14.00
" "	Lemon leaves; high-boron area; Oxnard	34.12	14.50

matter of lemon leaves from cultures receiving adequate potassium, the total boron was 134.1 p.p.m. of which 15.0 p.p.m. were water insoluble; in leaves from cultures receiving low potassium, the values were 240 and 19.1 p.p.m.,

TABLE X

EFFECT OF pH AND NITROGEN CONTENT OF THE SOIL ON THE WATER SOLUBILITY OF BORON IN THE DRY MATTER OF CITRUS LEAVES

VARIETY	NITROGEN FERTILIZATION	SOIL AMENDMENT ADDED	BORON IN DRY MATTER OF LEAVES	
			WATER-SOLUBLE	WATER-INSOLUBLE
	Equiv. N		*p.p.m.*	*p.pm.*
Valencia orange cuttings in 4000 gm. of Hanford soil	Calcium nitrate	Calcium carbonate to make 15%	7.74	15.23
	Ammonium sulphate	Sulphur, 2 gm.	17.50	16.53
	Calcium nitrate	Calcium carbonate, 20 gm.	7.50	13.43
	Ammonium sulphate	Sulphur, 1 gm.	20.35	16.75
Navel orange trees on sweet orange stocks in comparable plots on Romona loam soil at Citrus Experiment Station, Riverside	No nitrogen (check)	Cover crop	72.50	25.00
	Ammonium sulphate (3 lb. N per tree)	" "	45.46	23.70
	Calcium nitrate (3 lb. N per tree)	" "	43.83	18.46

respectively. For orange leaves from adequate potassium cultures the values were 136.4 and 15.1 p.p.m., respectively; whereas for leaves from low-potassium cultures the values 170.1 and 14.1 p.p.m., respectively, were obtained. These data do not lend support to the findings (**21**) that soluble boron in tissue is a function of the soluble potassium.

Summary

Gum was sometimes found in the peel of lemon and orange and also about the core in orange fruits produced by trees grown in solution cultures lacking boron. The vessels of the woody tissues in such trees were often filled with gum; exudations occurred at cut surfaces and in some cases the gum protruded through the bark. Gum was exuded from the exposed surface of the pedicel when flowers or fruits were detached. It also occurred between the cells in leaves and was involved in leaf distortion. Translucent spots frequently preceded the corky splitting of leaf veins and often the terminal meristematic tissue or growing points in twigs was destroyed.

Symptoms and chemical analyses are useful supplementary guides; no single symptom can be considered as characteristic or specific for boron deficiency or excess.

The turmeric method was found accurate and rapid for determining small amounts of boron in citrus. It permits the fractionation of the boron into soluble and insoluble portions in small samples and thereby avoids the interference of large amounts of gelatinous pectin.

Orange juice varied in its boron content according to the location of the trees from which the fruits were obtained. In comparison with orange juice, the boron content of grapefruit juice is low.

The boron content of Valencia orange fruits increased with age. The application of various amounts of boron to the soil increased the water-soluble boron content of the leaves of navel orange trees and of the fruit pulp. In dried citrus tissues, a large amount of the boron insoluble in distilled water was found to be soluble in weak acid.

Decreasing the pH of the soil or increasing the deficiency of other elements such as N and K increased the accumulation of boron in citrus leaves.

CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA

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EFFECT OF MECHANICAL STIMULATION ON THE ELECTRICAL AND CURVATURE RESPONSES IN THE AVENA COLEOPTILE

A. R. SCHRANK

(WITH THREE FIGURES)

Introduction

The almost innumerable experiments on the effects of auxins on plant growth have raised two challenging questions. (1) By what mechanism do the auxins exert their effect on the complicated process of growth? (2) What is the identity of the functional polarity that is responsible for the auxin transport? The present investigation is concerned primarily with the second of these questions.

It is generally accepted that the Avena coleoptile in the horizontal position will respond to gravity by bending upward (**2, 23**), and by establishing a transverse electrical polarity (**1, 3, 4, 5, 6, 19, 24**) oriented so that the electronegative, or upper side, becomes the concave side. It has previously been shown that this electrical polarity is established *before* any evidence of the unequal distribution of growth hormones occurs and before upward curvature begins (**18**).

STARK's (**20**) experiments have proved that the Avena coleoptile will respond to mechanical stimulation by bending toward the stimulated side. These experiments have been confirmed by SCHRANK (**17**). BROWN, LUND, and MARSH (**7, 14, 15**) have demonstrated that mechanical stimulation induces changes in electrical polarity in living plants such that the stimulated region becomes electronegative to the unstimulated region. Likewise, a transverse electrical polarity is established in the Avena coleoptile when it is mechanically stimulated on one side (**17**), and as in other plants, the orientation of this polarity is such that the stimulated side becomes electronegative to the unstimulated. Since the curvature in this case is toward the stimulated side, it follows, as in geotropically stimulated plants, that the negative side again becomes the concave side.

Finally, it has been shown that the upward curvature of the coleoptile in the horizontal position can be prevented by repeated mechanical stimulation of the under side which establishes a transverse electrical polarity opposite in orientation to the transverse electrical polarity established by gravity alone (**17**).

In the light of these facts, it becomes significant to inquire: (1) In the vertically oriented coleoptile, how does a variation in the duration of the applied mechanical stimulation affect the magnitude of the transverse electrical polarity that is established and the rate and magnitude of the resulting curvature? (2) What effect does mechanical stimulation of the coleoptile in the horizontal position have on the transverse electrical polarity and geo-

tropic curvature? (3) In what way does the removal of the apex, locus of origin of auxin in the coleoptile, affect the establishment of a transverse electrical polarity and growth curvature?

Apparatus and methods

The apparatus used in these experiments has previously been described (**17**). Only the coleoptile holder was slightly modified so that the plant could be held more securely in its position. The coleoptile was threaded through a hole slightly larger in diameter than the diameter of the coleoptile, in a small piece of lucite which was attached to the holder proper [fig. 1, (**17**)]. The coleoptile was inserted so that the apical 25 millimeters extended upward through the hole and fastened in this position by a small piece of moist cotton wedged between the lucite and the coleoptile.

All experiments were performed in a dark room under a neon light constructed out of ruby glass tubing (**17**). No means of controlling temperature was employed, but the variation was never greater than $\pm 0.25°$ C. during one experiment.

Pure line samples of Avena sativa (Victory Strain, C.I. 2020), which were obtained from the U. S. Department of Agriculture,[1] were used in all experiments. The seeds were husked, soaked, sprouted, and transplanted to individual containers in the usual manner. The seedlings were kept in the dark except for the sprouting period, during which they were kept under red light (60-watt General Electric, dark-ruby, photographic bulb) to inhibit the growth of the first internode. All of the plants used were intact coleoptiles 30 mm. ($\pm$ 1 mm.) in length and perfectly straight. The plants had a well-developed root system and leaves extending into the tip of the sheath; they were allowed a 30-minute rest period in the apparatus before beginning the experiment. During the 30-minute prestimulation period, the plants manifested active growth (7 ± 2 ocular scale divisions) and were still straight at the time of stimulation.

The electrical measurements were made with a direct current vacuum tube voltmeter with a bridge type circuit (**19**). This instrument was properly shielded and so adjusted that 1 millivolt gave a deflection of 1 millimeter on the scale. Readings were taken every minute, and the electrical zero of the instrument was kept constant by an occasional slight adjustment. Zinc-zinc sulphate electrodes that were never more than $\pm$ 0.5 millivolts from iso-electric were used. SHIVE's solution in tap water was the contact medium.

The use of mechanical energy for stimulation has two distinct limitations. First, it is extremely difficult to set up a device to stimulate a selected portion of a coleoptile with a uniform and measured intensity. Secondly, mechanical stimuli cannot be applied without bending or displacing the coleoptile from its original position. The mechanical stimuli in these experiments

[1] Appreciation is expressed to DR. T. R. STANTON of the U. S. Department of Agriculture.

were applied by an electrically controlled vibrator (**17**). This vibrator was mounted on a micromanipulator so that its position could be accurately controlled and duplicated from one experiment to the next. This was done to keep the intensity of the stimuli as uniform as possible. The mechanical displacement of the plants was kept at a minimum by using a vibrator with a very short amplitude.

For the electrical studies only point contacts were used, and they were always placed on opposite sides of the coleoptile on the short transverse axis 1.67 millimeters below the apex unless otherwise stated. Each contact is labelled with a lower case letter and the E.M.F. curves are plotted as in previous papers (**17, 18, 19**). Since the first letter used in labelling the E.M.F. curves always indicates the variable contact, and the second letter always denotes the earthed contact, all of the E.M.F. curves in figures 1 and 2 are *d-a* curves and those in figure 3 are *h-e* curves.

Curvature of the coleoptile was measured by an ocular micrometer in which each scale division equalled 1/18 millimeter. An India ink spot was placed at the very tip of the plant and the position of this spot recorded by use of the ocular. The lateral displacement of a point on the tip with respect to the longitudinal axis of the plant is not proposed as a method of measuring absolute curvature, but when the displacement of the plant tip is 2 millimeters or less, this method can be used for the comparison of one curvature with another without serious error.

Experimental

Since all of the E.M.F. and curvature measurements in form of curves to be presented in this paper are averages of 5 or 10 experiments, it becomes

TABLE I

ELECTRICAL RESPONSES IN MILLIVOLTS OF AVENA COLEOPTILES IMMEDIATELY AFTER BEING MECHANICALLY STIMULATED AND CURVATURE RESPONSES EXPRESSED IN OCULAR SCALE DIVISIONS OF THE SAME PLANTS ONE HOUR AFTER STIMULATION. SEE FIGURE 1

DURATION OF STIMULUS IN SECONDS		MAXIMUM ELECTRICAL RESPONSE IN MILLIVOLTS											
		EXPERIMENT										TOTAL	AVERAGE
		1	2	3	4	5	6	7	8	9	10		
		mv.	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*
A	4	4	3	30	9	9	10	4	6	12	13	100	10.0
	6	10	27	24	18	15	37	12	18	26	10	197	19.7
	8	25	51	27	28	18	25	11	40	18	21	264	26.4
DURATION OF STIMULUS IN SECONDS		CURVATURE IN OCULAR SCALE DIVISIONS 1 HOUR AFTER STIMULATION											
		EXPERIMENT										TOTAL	AVERAGE
		1	2	3	4	5	6	7	8	9	10		
		div.	*div.*	*div.*	*div.*	*div.*	*div.*	*div.*	*div.*	*div.*	*div.*	*div.*	*div.*
B	4	12	9	26	13	24	7	7	12	1	10	121	12.1
	6	11	34	8	17	18	20	15	18	25	16	182	18.2
	8	26	40	28	18	17	26	13	25	14	33	240	24.0

advisable to indicate the extent of the individual variations. In experiments of this type, individual variations in some features of the responses

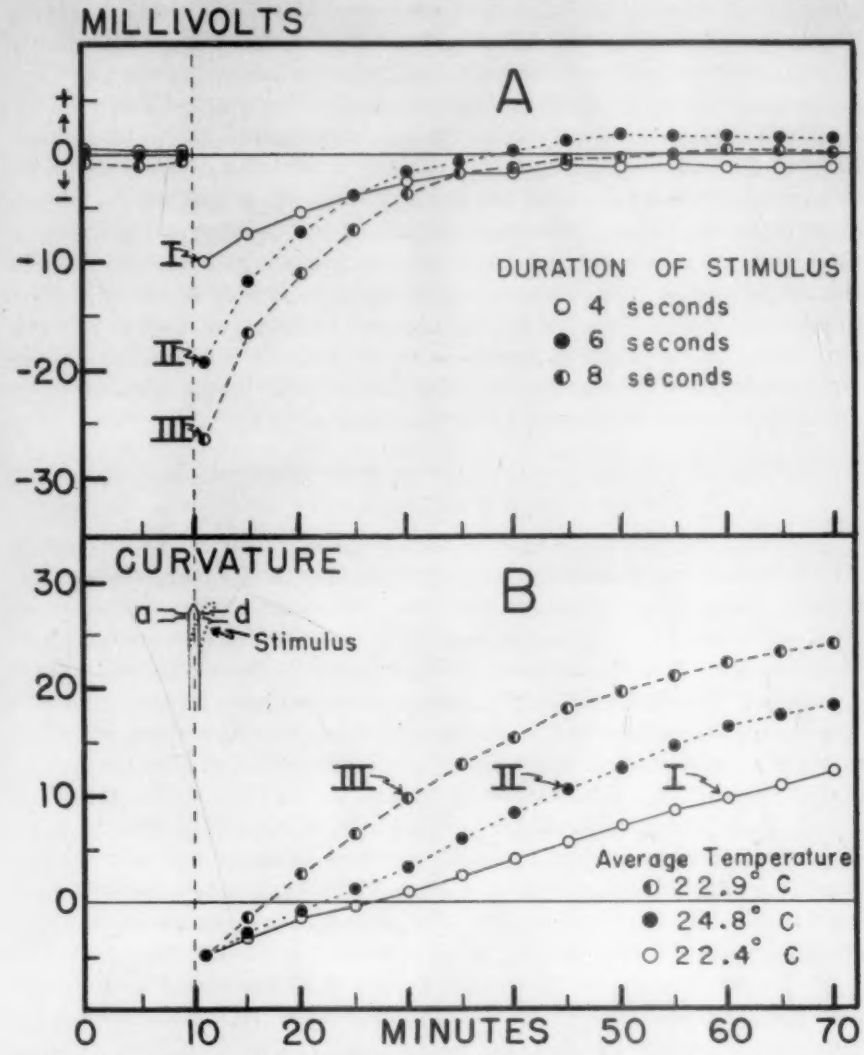

FIG. 1A. Transverse electrical polarities established by Avena coleoptiles 1.67 millimeters below the apex due to mechanical stimulation of the apical 10 millimeters of one side. Each curve represents the average of 10 experiments.

Curve I: 4-second stimulation
Curve II: 6 " "
Curve III: 8 " "

FIG. 1B. Corresponding average curvatures in scale divisions (18/mm.) of the plants in 1A.

are not uncommon; therefore, it might be expected that the electrical and curvature responses of different Avena coleoptiles to identical stimuli would

not always be the same. Even though the experimental plants are selected with extreme care, these variations still persist. All of the individual curves, both electrical and curvature, are remarkably similar in two respects. The shapes of all of the individual curves are much like the average curves; and the direction of every individual curve, with respect to the base line, is the same without exception as that indicated by the average curve.

The largest variation in the individual experiments comes in the magnitude of the electrical response immediately after stimulation and in the amount of curvature one hour after stimulation. In preparing the table to show the extent of the individual variations in the separate experiments these points of *maximum* variation were selected. Part A of table I shows the magnitudes of the electrical responses in millivolts of the individual coleoptiles immediately after the stimulus was applied, and Part B indicates the amount of curvature expressed in ocular scale divisions of each of the plants one hour after stimulation. The data in table I were taken from the experiments whose averages are shown in figure 1.

Effect of duration of mechanical stimulation on transverse electrical polarity and growth curvature

The data represented in figure 1 were obtained in the following manner. At the end of the 30-minute rest period the first E.M.F. and temperature readings were taken. Nine minutes later (39 minutes after the beginning of the experiment) the coleoptile elongation was recorded; the contacts were then removed, the position of the ink spot noted on the horizontally placed ocular scale in order to follow the subsequent curvature; and the mechanical stimulation device placed in position for stimulating only the apical 10 millimeters and so that the contracting surface of the vibrator was 10 scale divisions away from the coleoptile edge in each case. The amplitude of the vibrator was large enough to contact the coleoptile surface when the current was turned on. The vibrator was turned on so that the stimulus, regardless of its duration, would always end 30 seconds before the next reading was to be taken. The contacts were immediately replaced and the next electrical and curvature reading taken at the end of the 11th minute.

The curves in figure 1A represent electrical responses in millivolts. According to the contacts shown in the inset of figure 1B, all of the electrical curves are *d-a* curves in which the mechanical stimuli were applied to the *d* side. Every curve in figure 1 represents an average of 10 experiments. Although the potential readings were taken every minute, points are plotted at 5-minute intervals because it was found that such curves are representative. This is also true for all subsequent curves. Curve I in figure 1A shows the average electrical response of 10 coleoptiles, each one of which was mechanically stimulated for 4 seconds. In curve II the mechanical stimulus was applied for 6 seconds, and in curve III the plants were stimulated for 8 seconds.

The corresponding coleoptile curvature responses are shown in figure 1B. These curvatures are plotted in ocular scale divisions such that a positive

curvature represents a bending toward the stimulated side. These curves do not begin at 0 on the ordinate because the application of the mechanical stimulus bends the plants. It becomes significant to know whether or not the return of the coleoptile to the 0 position, which it occupied before the mechanical stimulus was applied, is curvature due to growth or due to the elasticity of the plant. Control experiments that were performed to evaluate this point indicate that the coleoptiles will return to the 0 position even when they have not been mechanically stimulated. In five experiments of this kind the plants were pushed 35 scale divisions beyond the 0 position with the micromanipulator in the same direction that they would be displaced by mechanical stimulation (toward the negative side of the graph in figure 1B) and held in this position for ten seconds. These plants were not stimulated mechanically as indicated by the fact that no electrical response was observed. All five of these plants returned to the 0 position in about 10 minutes, which is quite similar to the curves in figure 1B. However, none of these plants showed a positive curvature; they stayed in the 0 position rather than passing beyond it as did the plants represented by the curves in figure 1B. The fact that they did not manifest curvature can be taken as further evidence that they were not mechanically stimulated. These control experiments indicate that the return to the 0 position is probably not the result of growth curvature but rather a manifestation of elasticity.

Curve I in figure 1B represents the curvature of the same 10 plants whose average electrical response is shown in curve I in figure 1A. Curves II and III in figure 1B similarly correspond to curves II and III in figure 1A.

This series of experiments represented by figure 1 was repeated in a second very similar series. In the second series only the apical 5 millimeters of one side were mechanically stimulated rather than 10 millimeters as in the first. The results of the second series were essentially the same as those of the first, which helps to substantiate the results in figure 1 and indicates that mechanical stimulation of the apical 5 millimeters is practically as effective in inducing electrical and curvature responses as the same stimulation of the apical 10 millimeters. The data represented by the curves in figure 1 demonstrate the following facts: (1) The mechanically stimulated side of the coleoptile immediately becomes electronegative to the unstimulated side. (2) The magnitude of the electrical response is dependent on the duration of the mechanical stimulus applied. (3) Mechanically stimulating the apical 10 millimeters of one side of the coleoptile causes it to bend toward that side. (4) The rate and magnitude of the curvatures are dependent on the duration of the applied mechanical stimulus. (5) The rate and magnitude of the plant curvature can be approximately predicted from the magnitude of the electrical response and its rate of change.

Effect of mechanical stimulation on the transverse electrical polarity and growth curvature induced by gravity

Curve I in figure 2A shows the transverse electrical polarity that is established in coleoptiles at points 30 scale divisions below the apex when

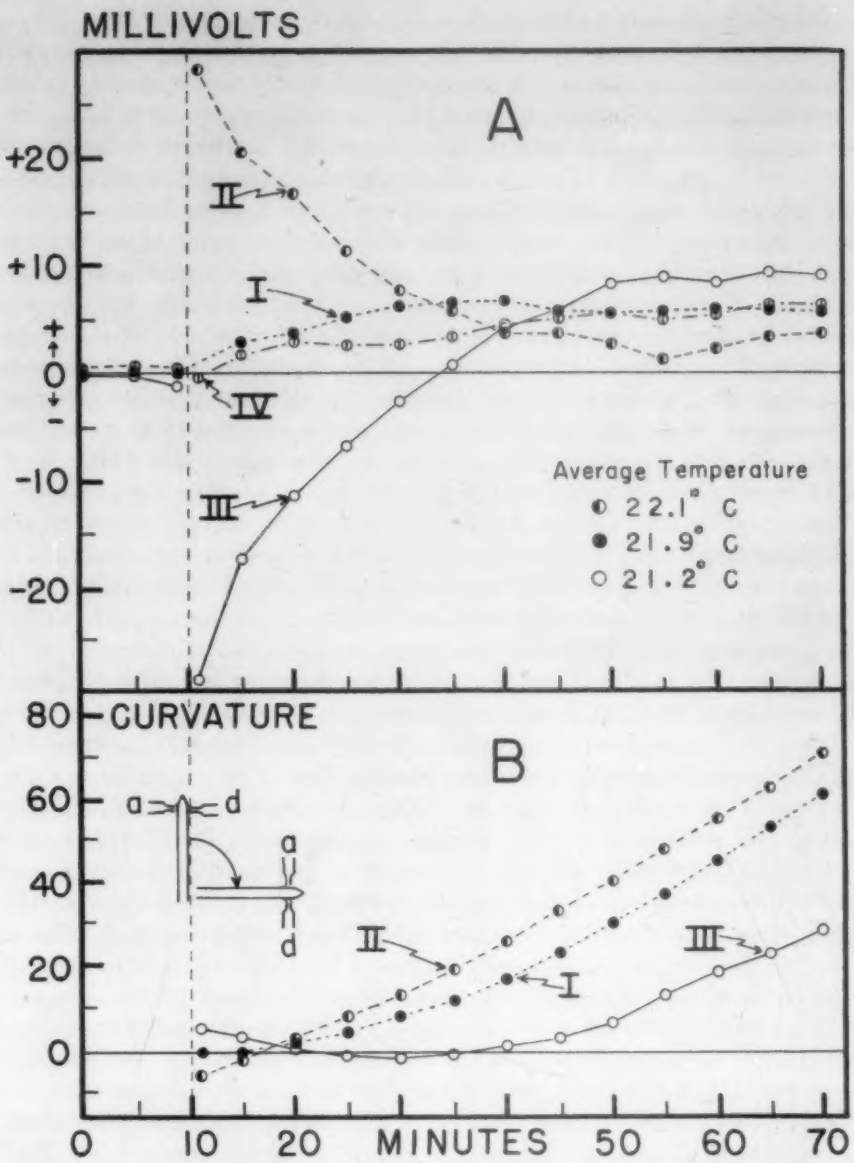

FIG. 2A. Transverse electrical polarities established by Avena coleoptiles 1.67 millimeters below the apex. Each curve represents an average of 5 experiments.

Curve I. Plants are changed from vertical to horizontal position.

Curve II. Plants are changed from vertical to horizontal position and a 10-second mechanical stimulus is applied to the apical 10 millimeters of the upper side.

Curve III. Plants are changed from vertical to horizontal position and a 10-second mechanical stimulus is applied to the apical 10 millimeters of the under side.

Curve IV. Calculated average of curves II and III.

FIG. 2B. Corresponding average curvatures in scale divisions (18/mm.) of the plants in 2A.

they are changed from the vertical to the horizontal position (control experiment). The curve represents an average of 5 experiments. The average upward curvature of these same plants is shown in curve I of figure 2B. These data corroborate previous work (**19**).

The E.M.F., curve II in figure 2A, is the average transverse electrical polarity established in 5 coleoptiles by a combination of mechanical and gravitational stimuli applied in the following manner: At the end of the ninth minute of the experiment the contacts were removed from the plants, the plants placed in the horizontal position, the apical 10 millimeters of the upper side stimulated with the mechanical vibrator for 10 seconds applied in the manner previously described. The mechanical stimulus was applied to the upper side in these experiments so that the mechanical and gravitational stimuli would be additive. It should be noted that this E.M.F. curve remains above the base line which means that the under side remains positive to the upper side for the duration of the experiment. Curve II in in figure 2B represents the corresponding upward curvature.

The experimental procedure used to obtain curves III in figures 2A and 2B was identical with the procedure used for curves II in the same figures in all but one respect: namely, the mechanical stimulus was applied to the under side of the coleoptile instead of the upper side. Even though the initial transverse electrical polarity recorded in curve II of figure 2A is reversed, with respect to the electrical polarity established by gravity alone (i.e., upper side electropositive to the under side), it does not remain so. The under side becomes electropositive 25 minutes after the mechanical stimulus was applied and remains that way for the duration of the experiment.

It should be noted that upward curvature begins 20 minutes after stimulation and before the under side becomes electropositive to the upper side. This should not be interpreted to mean that a transverse electrical polarity with the under side electropositive to the upper side is not essential for upward curvature, because the polarity in these experiments was determined only at points 30 scale divisions below the apex, while the entire coleoptile length contributes to the upward curvature. Earlier work (**19**) has demonstrated that a transverse electrical polarity is also established in the basal regions (25 millimeters below the apex) of coleoptiles when they are placed in the horizontal position. Since only the apical 10 millimeters were mechanically stimulated, it is very likely that the more basal unstimulated regions of the under side became electropositive to the upper side much earlier than in the apical regions.

Attention should also be called to an apparent quantitative discrepancy between transverse electrical polarities due to gravity and mechanical stimulation which is not paralleled by bending. The magnitude of the electrical polarity established by gravity is much smaller than the original electrical polarity established in the coleoptile by maximum mechanical stimulation (see curves I in figure 2A and III in figure 1A), but coleoptiles in the horizontal position bend more rapidly than plants that are kept in

the vertical position and stimulated mechanically (compare curve I in figure 2B with curve III in figure 1B). The data presented by these curves are not comparable in this respect because: (1) Only the apical 10 millimeters were stimulated mechanically while the entire 30 millimeters were stimulated by gravity. (2) The mechanical stimuli were applied for 8 seconds, while the force of gravity was in effect for 60 minutes.

It will be noted that the rates of curvature as represented by curves I and II in figure 2B become practically the same at about the same time that the corresponding electrical polarities become similar and stabilized. (Curves I and II in figure 2A.) This apparently means that the effects of the mechanical stimuli have disappeared, and that the plants are responding to gravity as the common stimulus.

The curves in figure 2 demonstrate the following facts: (1) Avena coleoptiles that are placed in the horizontal position establish a transverse electrical polarity with the under side electropositive to the upper side in the external circuit, and curve upward. (2) Plants that, in addition to being geotropically stimulated, have the apical 10 millimeters of the upper side mechanically stimulated, establish a larger initial electrical polarity and a faster rate of upward curvature than coleoptiles that receive only a geotropic stimulus. (3) Similar mechanical stimulation of the under side reverses the initial electrical polarity with respect to the electrical polarity induced by gravity and inhibits the upward curvature for 25 minutes. (4) Mechanical stimulation of the upper side does not increase the rate of upward curvature as much as the identical stimulation of the under side inhibits it.

One other interesting result comes from figure 2A. Theoretically it should be possible to take an average of curves II and III and duplicate curve I. Curve IV shows the results. The similarity in curves I and IV in this figure indicates that the method used for mechanically stimulating these plants is one that can be satisfactorily duplicated from one experiment to the next and might be considered a significant control for the experiments shown in figure 1.

An average of curves II and III in figure 2B does not duplicate curve I because, as previously stated, mechanical stimulation of the under side inhibits upward curvature more effectively than similar stimulation of the upper side accelerates it.

Effect of decapitation on transverse electrical polarity and growth curvatures

Since the growth hormones are produced in the extreme apex of the coleoptile (**21**), the hormone source can easily be removed by decapitation. Dolk (**10**) has shown that the growth rate of the Avena coleoptile reaches its minimum about 2½ hours after decapitation. It has long been accepted that growth of the coleoptile does not occur without growth hormones (**2, 23**), and it has recently been demonstrated that the growth hormone concentration is also lowest from 2 to 3 hours after decapitation (**22**). The

question raised in this connection is as follows: Is the transverse electrical polarity, which is established in the Avena coleoptile by a combination of mechanical and gravitational stimuli, dependent upon the presence of

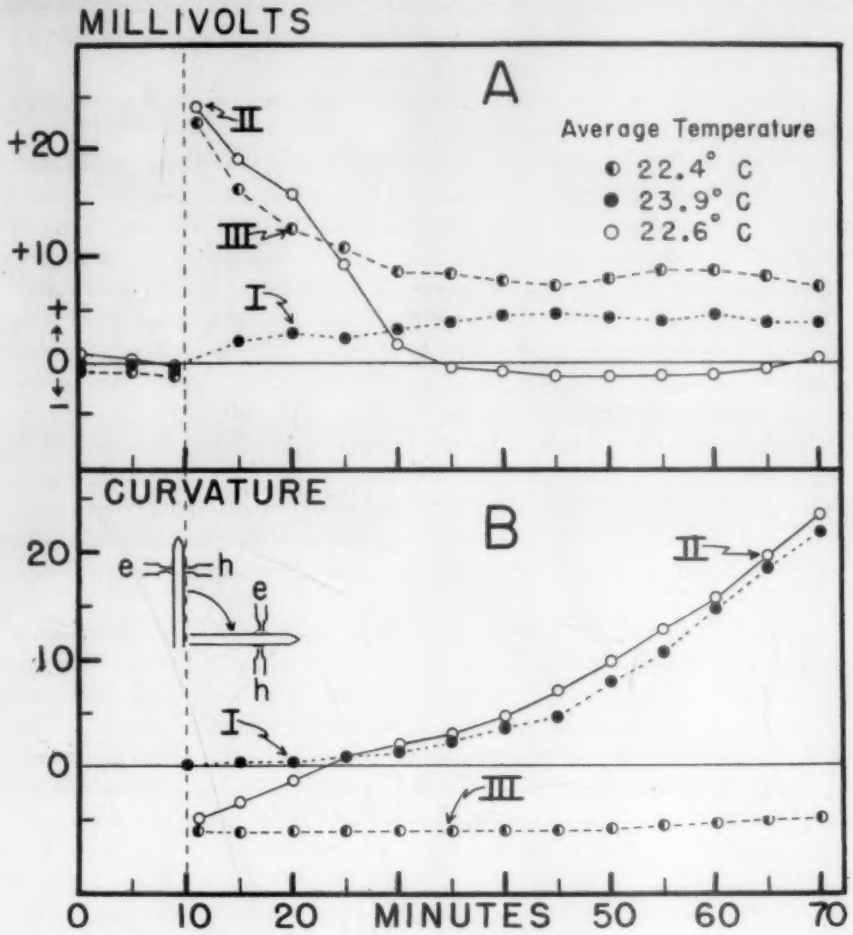

FIG. 3A. Transverse electrical polarities established by Avena coleoptiles 5 millimeters below the apex when stimulated as indicated below. Each curve represents an average of 5 experiments.

Curve I. Intact plants changed from vertical to horizontal position.

Curve II. Intact plants changed from vertical to horizontal position and a 10-second mechanical stimulation applied to the apical 10 millimeters of the upper side.

Curve III. Stimulated as in curve II. Plants had the apical 3 millimeters removed 2 hours and 10 minutes before stimulation. Stimulus applied to apical 7 millimeters.

FIG. 3B. Corresponding average curvatures in scale divisions (18/mm.) of the plants in 3A.

growth hormones in the plant? The data in figure 3 answer this question in the negative.

Curve I in figure 3A shows the transverse electrical polarity (average of

five intact plants) that is established 5 millimeters below the apex in Avena coleoptiles when they are changed from the vertical to the horizontal position. These data are consistent with earlier experiments (**19**). Curve I in figure 3B represents the upward curvature of the same five plants. The rate and total curvature are less in this curve than those represented in curve I in figure 2B because the curvature in these experiments was measured by observing the upward movement of an India ink spot placed 5 millimeters below the apex rather than at the tip.

Curve II in figure 3A shows an average of the transverse electrical polarity established 5 millimeters below the apex by 5 intact coleoptiles in response to a combination of mechanical and gravitational stimuli. The experimental procedure for the application of these stimuli was exactly the same as previously described for the data in curve II of figure 2A. The mechanical stimulus was applied to the upper side so that it would be additive with gravity. This was done in an effort to obtain a maximum electrical and curvature response. It should be noted that the E.M.F. curve crosses the base line after the plants have been in the horizontal position for 25 minutes. This means that the upper side becomes slightly positive to the under side which is directly opposite to the general observation (**19**). The significance of this reversal in electrical polarity is not apparent at the present time. Curve II in figure 3B shows the upward curvature manifested by these same 5 plants. For the reasons previously mentioned, this curve starts below the 0 line.

The E.M.F. curve III in figure 3A is an average curve from five coleoptiles that were treated exactly as those from which curve II in figure 3A was taken except that the apical 3 millimeters of these plants were removed with a razor blade 2 hours and 10 minutes before the stimuli were applied. The magnitude of the initial electrical response is approximately the same as the magnitude shown in curve II of figure 3A; however, in these experiments the under side remained positive to the upper side throughout the remainder of the period. Curve III in figure 3B represents the upward curvature of the same five plants. This average curve indicates that the plants begin to curve upward very slowly 40 minutes after having been stimulated. Only one of the plants included in this curve returned to the 0 position in the allotted 60 minutes. The fact that little or no upward curvature is shown by these coleoptiles is in agreement with earlier work (**2, 23**).

The data in figure 3 show these facts: (1) Gravity as the stimulus establishes a transverse electrical polarity of about 5 millivolts 5 millimeters below the apex of the Avena coleoptile and induces an upward curvature. (2) A combination of mechanical and gravitational stimuli induces a transverse electrical polarity and upward curvature. (3) The same combination of mechanical and gravitational stimuli induces a transverse electrical polarity but does not induce upward curvature in coleoptiles that have the apical 3 millimeters removed 2 hours and 10 minutes prior to stimulation. This can be interpreted to mean that a transverse electrical polarity can be established

in the absence of growth hormones. For a discussion of diffusible and bound auxin see WENT (**22**).

Discussion

In the evaluation of the data reported in this paper, the relationship between the magnitude of the transverse electrical polarity and the rate of curvature should be considered carefully. The nature of the experiments is such, especially those in which mechanical stimuli were applied, that the magnitude of the electrical polarity immediately after stimulation is of prime significance. This is true because the duration of the mechanical stimuli is relatively short and their electrical effects as measured do not last throughout the experiment. The fact that curvature continues even after the transverse electrical polarity disappears (fig. 1) does not indicate a discrepancy in the magnitude of electrical polarity and rate of curvature relationship. It must be remembered that the transverse electrical polarity was measured only at points 1.67 millimeters below the apex, that the apical 10 millimeters were mechanically stimulated, and that the zone of curvature migrates from the apex toward the base.

The CHOLODNY-WENT theory of growth curvature includes the necessity of a transverse "polarization" of cells to bring about an unequal hormone distribution (**8**). Experimental evidence at the present is inadequate to indicate what this "polarization" might be. Several investigators (**9, 11, 12, 13, 16**) have suggested that potential gradients might be adequate to displace the growth substances, but so far their experiments have not conclusively verified their suggestions. It has been shown that a transverse electrical polarity of several millivolts is established in the Avena coleoptile *prior* to an unequal hormone distribution or geotropic bending (**18**). STARK (**20**) demonstrated that the Avena coleoptile will bend toward the side that is mechanically stimulated. These experiments have been verified by SCHRANK (**17**) who has also shown that the mechanical stimulation which results in curvature previously establishes a transverse electrical polarity oriented so that the stimulated, or the electronegative, side becomes the concave side. This orientation is the same as in plants stimulated by gravity. Furthermore, it has been proved that the upward curvature of an Avena coleoptile in the horizontal position can be inhibited by mechanical stimulation of the apical 10 millimeters of the under side. This same mechanical stimulation establishes a transverse electrical polarity which is oriented opposite to the potential gradient established by gravity. This is not an injury phenomenon because similar stimulation of the upper side does not inhibit upward curvature (**17**). It should be remembered in this connection that mechanical stimulation of the upper side does not reverse the geoelectrical polarity but rather enhances it. The experiments in figure 1 clearly show that, within a certain range, the magnitude of the transverse electrical polarity that is established, its rate of change, and the subsequent rate of curvature are directly dependent on the duration of the stimulus. Actually, one could say dependent on the number of mechanical

stimuli applied; because, for example, in the 8-second stimulus the plant receives exactly twice as many taps as in the 4-second stimulus.

Curves I and IV in figure 3A verify the statement made in an earlier paper (**17**) : that mechanical stimuli applied to the upper side were additive with gravity, and similar stimuli applied to the under side were antagonistic to gravity as the stimulus, in so far as electrical and curvature responses are concerned.

It is quite commonly accepted that growth curvatures are dependent on the presence of the apex (**2, 23**). If the transverse electrical polarity is responsible for lateral hormone transport, it then seems reasonable that its establishment should *not* depend on the presence of growth hormones. The data in figure 3 show that a transverse electrical polarity can be established in the Avena coleoptile by a combination of gravitational and mechanical stimuli when the apical 3 millimeters of the plant are not present. The fact that these plants exhibit no upward curvature may further be taken to mean that no growth hormones were present (**22**).

In all of the facts that have just been presented there is not one that apparently contradicts the WENT-KÖGL scheme of "electrophoretic" auxin transport. Neither do any of these facts demonstrate a causal relationship between an electrical polarity and growth hormone transport. The crucial experiments to establish such a relationship have not as yet been performed. Meanwhile, there is no reason to assume that an electrical polarity, transverse or otherwise, does not play some other rôle in the linkage between growth hormones and growth. For example, it is theoretically possible that a potential difference of a certain orientation and magnitude across the cell could be a prerequisite condition for making the growth regulating mechanism receptive to the auxins which are already present within the cell. The validity of this suggestion remains to be tested.

Summary

1. The Avena coleoptile responds to mechanical stimuli applied to one side of the apical 10 millimeters by establishing a transverse electrical polarity oriented so that the stimulated side becomes electronegative in the external circuit to the unstimulated side, and by bending toward the electronegative and stimulated side. The magnitude of this transverse electrical polarity, its rate of change, and the rate of growth curvature are dependent on the duration of the mechanical stimulus.

2. Mechanical stimulation of the apical 5 millimeters has essentially the same effect as similar stimulation of the apical 10 millimeters.

3. Changing Avena coleoptiles from the vertical to the horizontal position causes them to establish a transverse electrical polarity with the under side electropositive to the upper and then bend upward. Simultaneous mechanical stimulation of the apical 10 millimeters of the upper side establishes a transverse electrical polarity of greater magnitude than gravity alone and also increases the rate of upward curvature. Similar mechanical

stimulation of the under side establishes a reversed (opposite to the polarity established by gravity) electrical polarity, inhibits upward curvature for 25 minutes, and decreases the subsequent rate of upward bending.

4. Avena coleoptiles that have had the apical 3 millimeters removed 2 hours and 10 minutes before a combination of mechanical and gravitational stimuli are applied establish a transverse electrical polarity 5 millimeters below the apex of approximately the same magnitude as undecapitated plants similarly stimulated. The fact that the decapitated plants manifest no upward curvature indicates that the presence of growth hormones is not essential for the establishment of a transverse electrical polarity.

5. All of the facts from the experiments presented are compatible with the WENT-KÖGL "electrophoretic" growth hormone transport theory, but they do not demonstrate a causal relationship between the electrical polarity and auxin transport.

DEPARTMENT OF ZOOLOGY AND PHYSIOLOGY
UNIVERSITY OF TEXAS
AUSTIN 12, TEXAS

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EXTENT AND LONGEVITY OF THE SEMINAL ROOTS OF CERTAIN GRASSES[1]

J. E. Weaver and Ellen Zink

(with seven figures)

Introduction

Grasses possess two distinct root systems. The primary or seminal root system begins development immediately upon the germination of the seed and consists of one to several main roots and their branches, the number varying with the species. The young plant is entirely dependent upon this primary root system for water and soil nutrients. Later, especially during the period of tiller production, a secondary or nodal root system develops from the lower nodes of the parent culm and from the tillers. The seminal roots are often designated as temporary in general texts and even in special books on grasses. Hitchcock (**1**) states: "The primary root persists only a short time after germination, its place being taken by secondary roots produced from the nodes of the young culm."

It has been observed by Locke and Clark (**5**) that where extremely dry surface soil or soil drying or crusting prevented normal development of nodal roots, the seminal roots furnished sufficient moisture to maintain the growth of the wheat plant to maturity. Weaver, Jean, and Crist (**13**), after numerous investigations on the root systems of several cereal crops, concluded that the seminal roots remain alive and active until the time of harvest. Simmonds and Sallans (**12**) found that the wheat plant may produce seed when dependent almost entirely upon the seminal roots. Simmonds (**11**) states that the seminal roots of wheat remain functional throughout the entire life of the plant. Records on absorption of water and salts from water cultures by Krassovsky (**3**) have shown that the seminal roots of wheat, barley, and rye are active up to the time of harvest. These findings were later confirmed by plants grown in soil (**4**). Pavlychenko (**9**) states that in the several annual grasses studied in arid climates, seminal roots functioned throughout the entire growing season and were frequently the only roots supporting the plant from emergence to maturity.

Studies on seminal roots of perennial grasses, however, are very few, and usually mention of their development is only incidental. The purpose of this study was to ascertain the extent of their development among perennial grasses; how long they remain alive and functional after the secondary root system is established and how much growth grasses can make when supported by the seminal roots alone.

Methods

Wooden boxes 10×10 inches in inside width and 24 inches deep were used. They were lined with galvanized iron. One side was removable. It

[1] Contribution from the Department of Botany, University of Nebraska, no. 145.

was held in place by means of two large iron bands and small bolts. The boxes were filled with loam potting soil, previously screened, brought to an approximately uniform water content favorable for rapid growth, and thoroughly mixed. In filling the boxes to within an inch of the top, the soil

TABLE I

DEVELOPMENT OF SHOOTS AND ROOT SYSTEMS OF 14 DIFFERENT NATIVE AND INTRODUCED GRASSES WITH DEGREE OF DECORTICATION OF SEMINAL ROOTS AT DIFFERENT PERIODS OF GROWTH

	Agropyron cristatum (L.) Beauv.				*Agropyron intermedium* (Host) Beauv.			
Age, days	21	41	53	90	21	37	59	96
Av. ht., in.	4.5	10	5	9	5.5	10.5	9	15
Av. no. lvs.	5	6	5	6	4	5	6	7
No. tillers	0	2–7	1–6	12–21	0	2–4	3–4	9–15
No. nodal roots	1–2	5–11	3–9	17–33	2–3	6–8	6–10	36–45
Depth, seminals, in.	7–9	10–22.5	12–15	20.5–23	7–9	18–23	16–23	20–26
Cortex, main root	Intact below 3 inches	Intact below 6 inches	Intact below 4 inches	Intact below 10–15 inches	Intact below 2 inches	Intact below 7 inches	Intact below 4 inches	None
Cortex, branches	Intact	Intact	Intact	Intact below 2 inches	Intact	Intact	Intact	Intact below 4 inches
Root hairs	Abundant at all examinations				Abundant at all examinations			
	Agropyron smithii Rydb.				*Andropogon furcatus* Muhl.			
Age, days	21	41	74	117	21	41	70	110
Av. ht., in.	3	10.5	16	19	1.5	8	8	18
Av. no. lvs.	2	5	7	9	5	7	8	11
No. tillers	0	0	0–2	4–7	0	1–3	1	5
No. nodal roots	1	3–4	6–12	25–32	0–1	4–6	2–10	10–22
Depth, seminals, in.	6–8	16–22.5	10.5–16	19–23	6–8	9–13	10–11	10–14
Cortex, main root	Intact	Intact on lower 6 inches	Intact on lower half	None	Intact below 4 inches	Partially absent except near ends	None except at tips	None except at tips
Cortex, branches	Intact	Intact	Intact	Intact on many finer branches	Intact	Intact except on proximal ends of longer ones	Intact	Nearly intact
Root hairs	Abundant at all examinations				Usually only moderately abundant			

was tamped firmly in place. The top three-fourths inch consisted of loam soil intermixed with an equal volume of sand.

One box was used for each species of grass. The seeds were planted shallowly about 3 inches from each corner of the box and also in the center. Proper watering and mulching promoted prompt germination and good establishment. The seedlings were later thinned to 5 or fewer plants in each of the five areas. Water was applied sparingly so that the soil never became

too wet. When roots were to be examined on plants only about three weeks old, no further preparation was necessary since the seminal root could readily be distinguished from nodal roots. But when examination was to be

TABLE I (*Continued*)

DEVELOPMENT OF SHOOTS AND ROOT SYSTEMS OF 14 DIFFERENT NATIVE AND INTRODUCED GRASSES WITH DEGREE OF DECORTICATION OF SEMINAL ROOTS AT DIFFERENT PERIODS OF GROWTH

	Andropogon scoparius Michx.				*Bouteloua curtipendula* (Michx.) Torr.			
Age, days	21	41	73	110	21	37	70	118
Av. ht., in.	1.5	8	9	14	3.5	11	9	18
Av. no. lvs.	5	6	7	9	6	6	5	8
No. tillers	0–1	3–7	2–5	4–11	0–1	3–4	3–4	8
No. nodal roots	1–2	5–7	6–9	18–32	2–3	3–7	5–11	30–38
Depth, seminals, in.	6–8	10–14	11–12	8–12	9–10	14–16	10–15	13–16
Cortex, main root	Intact	Intact below 3 inches	Intact below 2 inches	Nearly intact	Intact below 3 inches	Intact below 6–8 inches	Intact below 5 inches	None
Cortex, branches	Intact	Intact	Intact	Intact	Intact	Intact almost throughout	Intact almost throughout	Intact on some; on others at distal end only
Root hairs	Fairly abundant	Not abundant			Abundant	Abundant	Infrequent on main root, abundant on branches	Present

	Bromus inermis Leyss.				*Buchloe dactyloides* (Nutt.) Engelm.			
Age, days	21	37	61	108	21	41	74	123
Av. ht., in.	5	9	14	16	2	6	4.5	6
Av. no. lvs.	3	5	8	8	5	7	5	8
No. tillers	0	0–1	1–5	Many	0–2	2–11	2–17	17–21
No. nodal roots	2–3	2–6	16–25	Many	0	9–14	4–12	22–33
Depth, seminals, in.	7–8	15.5–23	17–23	18–24	7–8	7.5–14	10–11	12–14
Cortex, main root	Intact	Intact below 6 inches	Intact on lower half	None except on lower 2–6 inches	Intact on lower 2 inches	Intact only on last inch	Intact only near tip	Intact only near tip
Cortex, branches	Intact	Intact	Usually intact	Intact on many; on distal parts of others	Intact	Intact on small branches		
Root hairs	Abundant at all examinations				Abundant at all examinations			

made after 6 to 17 weeks of growth, the seminal roots were banded when about 3 weeks old. In a few grasses where there was more than one seminal root, the one of earliest emergence and greatest length was banded. Banding was facilitated by tilting the box so that after removing the mulch the

layer of sandy soil could be carefully washed away with water from a bulb type of plant spray. A small band of very thin, pure sheet tin about 2 mm. wide was gently but rather tightly rolled about each seminal root near its origin. About 20 plants were thus banded in each box, care being taken to

TABLE I (*Continued*)

DEVELOPMENT OF SHOOTS AND ROOT SYSTEMS OF 14 DIFFERENT NATIVE AND INTRODUCED GRASSES WITH DEGREE OF DECORTICATION OF SEMINAL ROOTS AT DIFFERENT PERIODS OF GROWTH

	Elymus canadensis L.				*Elymus junceus* Fisch.			
Age, days	21	41	73	112	21	42	71	110
Av. ht., in.	3	6	10	14	3.5	8	11	11
Av. no. lvs.	3	5	5	7	3	5	6	7
No. tillers	0	3–7	2–5	8–14	0	2–3	2–4	14
No. nodal roots	0–1	9–12	8–21	33–60	2	5–9	6–15	23–44
Depth, seminals, in.	6–7	16–18	17–24	18–25	7.5–8	8–14	15–23	20–23
Cortex, main root	Intact	Intact	Intact	Intact	Intact	Intact only on last 5 inches	None	None
Cortex, branches	Intact	Intact	Intact	Intact	Intact	Intact below 3 inches	Intact	Only on finest branches and tips of branches
Root hairs	Present	Present	Present	Abundant on branches	Abundant at all examinations			
	Panicum virgatum L.				*Sorghastrum nutans* (L.) Nash			
Age, days	21	37	73	108	22	41	71	110
Av. ht., in.	3	8	16	19	4	15	9	14
Av. no. lvs.	4	6	6	7	4	5	6	7
No. tillers	0	0–3	3	2–12	0	2–3	2–4	9–15
No. nodal roots	2–3	4–5	6–9	21–28	2–4	6–12	5–11	25–40
Depth, seminals, in.	7–8	9–17	12–14.5	11.5–23	7–8.5	9.5–15	10–11	7.5–15
Cortex, main root	Intact	Intact	Intact	Intact	Intact	Intact below 10 inches	Intact on lower half	None
Cortex, branches	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Mostly intact
Root hairs	Usually abundant on branches, fewer on main root				Sparse on main root, abundant on laterals	Infrequent on both main root and laterals	Infrequent	Irregularly distributed; locally abundant

keep the roots moist while banding and to cover them immediately afterward with a dry sandy soil which was then watered.

Examination of the seminal root was made after the side of the box had been removed. Sometimes it was necessary to tilt the box backward to pre-

vent the soil from caving. In earlier stages of growth root excavation was accomplished rather easily with the aid of an ice pick. The main root and all of its laterals were exposed and the depth and lateral spread recorded. Often the entire seminal root system was obtained intact. Usually only the plants in the front of the box were removed during the first examination. The soil that had been removed was replaced by a new lot of soil firmly tamped, after the side and bands had been replaced. The remaining plants continued growth unharmed. But with older plants root examination became more difficult and often extremely so since not only were the seminal roots longer and branched more extensively but also they were intermixed

TABLE I (*Concluded*)

DEVELOPMENT OF SHOOTS AND ROOT SYSTEMS OF 14 DIFFERENT NATIVE AND INTRODUCED GRASSES WITH DEGREE OF DECORTICATION OF SEMINAL ROOTS AT DIFFERENT PERIODS OF GROWTH

	Stipa comata Trin. AND Rupr.				*Stipa spartea* Trin.			
Age, days	21	42	74	123	21	41	73	113
Av. ht., in.	1.5	6.5	4	5	5	13	8	14
Av. no. lvs.	3	4	5	7	2	5	5	6
No. tillers	0	1–2	1–3	6–8	0	0	0–1	1–3
No. nodal roots	0–1	3–4	3–5	6–10	2	3–4	22–24	7–10
Depth, seminals, in.	7.5–8	17.5–27	20–24	20–24	7–8	23–24	4–7	23.5–25
Cortex, main root	Intact	None except near tip	None	None	Intact below 3 inches	Intact only on lower 4 inches	Intact on lower half	Intact only on lower 2–4 inches
Cortex, branches	Intact	Intact	Intact	Intact below 5 inches	Intact	Intact	Intact	Intact except on proximal portions of larger ones
Root hairs	Abundant at all examinations				Abundant at all examinations			

with the rapidly developing and numerous roots of the secondary or nodal root system. Often gentle washing with the bulb spray was used as a supplementary method. Sometimes, despite great care, roots were broken, but always at least several of the twenty available for examination were traced to their ends. Ample material was always available for microscopic examinations which revealed the presence or absence of root hairs and the places and extent of decortication.

Two separate plantings were made; one in spring, which furnished materials for examinations at ages of approximately 21 and 41 days after planting, and another one in late fall. In the second lot of plants the seminal roots were examined at ages of about 70 and 119 days. In spring and summer the seedlings grew in a greenhouse with panels of glass removed from the sides and with windows and ventilators open so that the wind blew through the house. Light was about 40 to 50 per cent. that of full sunshine.

In fall and winter usual greenhouse conditions prevailed except that artificial lighting extended the light day until 10 P.M.

Ten species of native grasses and four species of introduced grasses of great economic importance were grown. Supplementary experiments included wheat and oats. The 14 species are listed in table I where the results of the first experiment are tabulated.

Results

Conditions of seminal roots at different ages

Data in the first two columns under each species in table I are from seedlings planted on May 11 and examined approximately 21 and 41 days later. Conditions for growth were very favorable and the plants developed rapidly. Data in the last two columns are from plants grown from seed sown on, or shortly after, October 21 and examined after approximately 73 and 119 days. Obviously all could not be examined even within the same week; the exact number of days of growth is given for each species. Growth was slower than in the spring, but usually development was further advanced at the first examination of plants started in fall than at the second examination of plants grown in spring.

The data are the average of at least 5 plants and often of 8 to 10 of each species; several plants were examined in order to obtain a composite view of the development and condition of the root systems. The number of leaves is that of the parent culm only. The number of nodal roots is included since it is not only a good criterion of the degree of development of the individual but also reveals something of the importance of the secondary root system in comparison with the primary one. Although the seminal roots usually, but not always, grew more or less vertically downward, their extent is given in depth of soil penetration rather than in length. The cortex is described as intact only when microscopic examination revealed that there was no sloughing below the depth stated. Its cells appeared clear and turgid; the root hairs on it were also apparently normal and functioning. Sometimes large areas of the main root above the intact level also possessed normal and functioning cortex; more often it occurred only in local areas or patches. Normally degeneration of the cortex progressed regularly from the proximal toward the distal end of the main root. Near the root tips usually 1 to 2 inches of cortex remained intact throughout the experiment. Decortication on the branches proceeded in a similar manner but much more tardily than on the main root. The abundance or paucity of root hairs indicated in table I refers only to those portions of the root system with cortex still intact.

First examination.—Twenty-one days after planting, when the seedlings were between 1.5 and 5.5 inches high and rarely had a tiller, the roots of all species were between 6 and 9 inches deep. An exception was that of *Bouteloua curtipendula* where they were 9 to 10 inches deep, a fact probably correlated with its very rapid germination. One to 4 nodal roots were present on all grasses except *Buchloe dactyloides*. The cortex was intact in 8

species and absent from the main root only above 2 to 4 inches depth in the others, except for one where it occurred only on the two inches immediately above the tip. This early examination afforded an excellent background for the later studies.

SECOND EXAMINATION.—The grasses were mostly 8 to 10 inches tall and in the fifth- to sixth-leaf stage after 41 days. All but *Agropyron smithii* and *Stipa spartea* had 1 to 4 tillers and certain species as many as 7 to 11. Number of nodal roots had increased greatly. The range in number was from 3–4

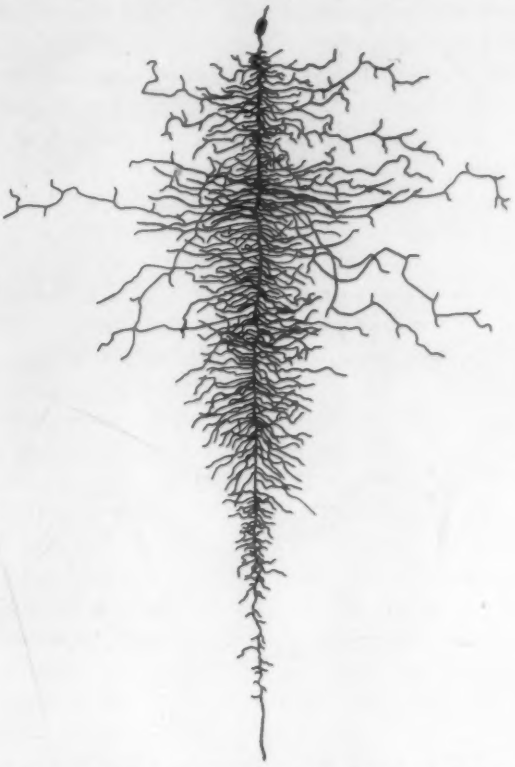

FIG. 1. Seminal root system of *Panicum virgatum* on May 13 grown from seed planted 63 days earlier. The top, which was nearly a foot tall and had 6 leaves, was supported entirely by the seminal root which was 12 inches long, the nodal roots having been repeatedly excised.

to 9–14 and in many of the boxes some of these roots had extended to the bottom. The pattern of the seminal root system, except that the roots were much finer, was in general not greatly unlike that of other grass roots. But as in other root systems, the details of the pattern varied widely among the species. A few examples will illustrate some differences. The light-colored and relatively coarse seminal root of *Panicum virgatum* was always dominant and its branches subordinate. The lateral spread was relatively small (fig. 1). The dark-colored and extremely fine seminal root of *Buchloe*

dactyloides held its course definitely, penetrated downward nearly vertically, and was not easily confused with its major branches. The dark-colored seminal root of *Andropogon furcatus* branched widely not only in the surface soil but also at greater depths (fig. 2). Conversely, the light-colored seminal root of *A. scoparius,* while profusely branched throughout its course, had, except in the surface 2 inches of soil, branches that were rarely over 1.5 inches long. The seminal roots of *Stipa comata* had relatively few long branches and even short ones were not abundant. *Bouteloua curtipendula* had seminal root branches which were very long and extremely fine, often being cobweb-like in appearance. The seminal roots of *Agropyron intermedium* were also extremely fine, often being almost invisible against the background of dark-colored soil. The quality of fineness of the seminal roots aided greatly in preventing confusing them with portions of nodal roots.

The seminal roots, when measured one-half inch below the seed, varied in diameter with the species and with decortication from 0.3 to 0.1 mm. Exceptions were roots of *Sorghastrum nutans* (0.33 mm.) and *Stipa spartea* where a diameter of 0.6 mm. was found. In some grasses the seed coat remained in place for a long time and continued to show clearly the place of origin of the seminal root. *Stipa spartea* and *Bouteloua curtipendula* are examples. The seed coats of *Buchloe dactyloides* and certain others were still undecayed 73 days after planting.

The shallowest seminal roots when 41 days old were those of *Andropogon scoparius, A. furcatus, Buchloe dactyloides,* and *Elymus junceus.* They were only 7.5 to 14 inches deep. The deepest were those of *Agropyron intermedium, Stipa spartea,* and *S. comata,* which either extended to or ran along the bottom of the box. Some nodal roots of all species were abundant in the first foot of soil and in most species they also reached the maximum depth of the soil. The cortex on the main root was either entirely intact (2 species), intact below 3 to 10 inches depth (6 species), or absent except on 1 to 6 inches of the distal end, as in *Agropyron smithii,* both species of *Stipa, Andropogon furcatus, Buchloe dactyloides,* and *Elymus junceus.* None of the branches were decorticated, except a small part on their proximal ends in *Andropogon furcatus* and *Elymus junceus,* but only the smaller branches of *Buchloe dactyloides* retained the cortex.

THIRD EXAMINATION.—The age of the plants at this examination ranged from 61 to 74 days, except that of *Agropyron cristatum* and *A. intermedium* which was 53 and 59 days old, respectively. Grasses of intermediate height were 8 to 11 inches tall. A few, including *Buchloe dactyloides* and *Stipa comata,* were only 4 to 5 inches high, but others, as *Bromus inermis,* were 14 to 16 inches tall. The leaf-stage ranged from the fifth to the eighth. Plants of all species had at least 1 tiller, 2 to 4 were common, but *Buchloe dactyloides* possessed 2 to 17. In five of the boxes the tillers were about two-thirds as tall as the parent plant. In five, they were about half as tall; but those of *Agropyron intermedium* and *Bromus inermis* were as high as the parent culms. Many new nodal roots had developed. The fewest were 3 to 5

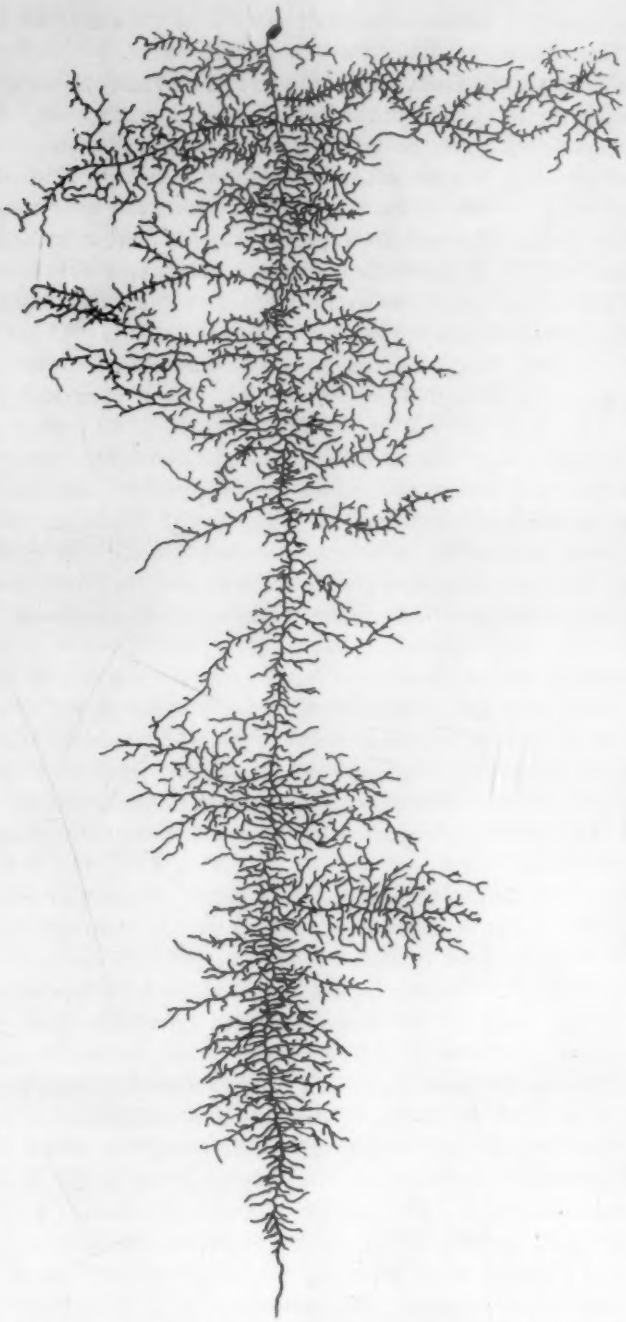

FIG. 2. Seminal root system of *Andropogon furcatus* on May 20, grown from seed planted 70 days earlier. The depth is 21 inches. The top grown on this root alone is shown in figure 3.

(*Stipa comata*); 6 to 10 were frequent; but some plants of *Elymus canadensis* and *Bromus inermis* had 21 and 25, respectively.

Depth of root penetration varied from 10 to 24 inches. Some species were no more deeply rooted than at the second examination in spring. The cortex on the main root of *Elymus canadensis* and *Panicum virgatum* was intact; on four other species it was intact below 2 to 5 inches. But on *Elymus junceus* and *Stipa comata* there was none. It occurred only near the root tip in *Buchloe dactyloides* and *Andropogon furcatus*, but over the lower half of the root of plants of four other species. Cortex of the branches was entirely or almost entirely intact on all but *Buchloe dactyloides*, where it remained regularly only on the smaller branches.

FOURTH EXAMINATION.—At the final examination the various grasses ranged in age from 90 to 123 days (table I). They were well developed. Many were 9 to 15 inches tall but several were 18 to 19 inches in average height. The number of leaves had increased; there were mostly 7 to 9. Except for the sod-forming *Agropyron smithii* and the slow-growing *Stipa spartea*, the number of tillers ranged from 5–8 to 17–21 per plant. The abundant tillers were often as tall as the parent culm. *Bouteloua curtipendula* and *Panicum virgatum* were in blossom and the bunches of *Buchloe dactyloides* with stolons 4 to 20 inches long had the appearance of mature plants.

This examination was the most difficult since the number of roots of the secondary root system had greatly increased. Twenty to 30 roots per plant were common, except for species of Stipa where 6 to 10 prevailed. Plants of *Elymus canadensis* and *Agropyron intermedium* possessed 33 to 60 each. Most of the seminal roots now extended deeply and frequently to the bottom of the box. They were intermixed with the nodal roots throughout much or most of their course.

The cortex was intact throughout in *Elymus canadensis* and *Panicum virgatum*, nearly intact in *Andropogon scoparius*, and not degenerated below 10–15 inches in *Agropyron cristatum*. It occurred only on the lower 1 to 6 inches of the roots of *Andropogon furcatus, Bromus inermis, Buchloe dactyloides*, and *Stipa spartea*. On the six remaining species there was none. Cortex on the branches was nearly or entirely intact on the first four species noted above, where the same condition prevailed on the main root of three of these species. This condition of the branches maintained also in *Agropyron intermedium, Andropogon furcatus, Sorghastrum nutans*, and *Stipa comata*. Conversely, cortex was found only on many of the finer branches of the remaining species. The surface of the main seminal root composed only a very small portion of the entire absorbing surface of the greatly branched root system. Even if it and the proximal portions of the major branches were decorticated the root probably could still absorb and transport water and solutes at nearly its former capacity.

Judging from the macroscopic appearance of the relation of the seminal roots to the soil, in conjunction with extensive examinations of numerous

portions of the roots under the microscope, it appeared that the extent of physiological activity in the several species at this last examination was quite different. In *Elymus junceus* it seemed that all of the roots were nonfunctional as absorbing organs except near the root tips. Perhaps only one-third of the entire root surface was active in absorption in *Agropyron intermedium*, *A. smithii*, *Bouteloua curtipendula*, and *Buchloe dactyloides*. It seemed clear that the absorbing surface was considerably greater in the remaining species where one-half to two-thirds of the entire root system appeared to be intact. Much information was gained about longevity and activity of the seminal roots in later experiments in which development of the secondary root system was prevented and the plants were supplied with water and nutrients by the seminal roots alone.

Maximum depths of penetration

Since many of the seminal roots reached the bottom of the boxes, a new lot of each of four species of grasses was grown in boxes of the same surface area but 4 feet deep. Seeds were planted in March or April and the seminal roots were banded when the seedlings were in the second-leaf stage. The seminal roots of *Stipa comata* 77 days after planting were growing vigorously and showed no decortication. Depths of penetration varied from 17 to 20.5 inches on five plants. Some roots of the secondary root system penetrated to about the same depth. Roots of *Agropyron intermedium* when 70 days old were traced to depths of 32 to 36 inches. After 46 days the seminal roots of *Panicum virgatum*, which were in excellent condition throughout and apparently still elongating, were 18.5 to 19 inches deep.

From the foregoing experiments it may be seen that the seminal roots of these grasses reached depths of 2 to 3 feet, although some did not extend much deeper than 1 foot, at least during the first 70–119 days of their growth.

Development of plants grown on the seminal root

Grasses of each of four species were grown on the seminal root system alone, roots of the secondary system being repeatedly excised. The seedlings were grown in boxes of soil as described and the seminal roots were banded after they were established. A flat cork, 2.25 inches in diameter and one-fourth inch thick, with a small hole in the center was partly cut and then broken into halves and inserted tightly about the root just below the band, the root occupying the central opening. The cork was held together by a copper wire, and fastened in place in the soil by two long nails inserted through holes on opposite sides from the center. Corks and roots were then covered with a mixture of sand and loam which was kept moist by daily sprinkling. The cork furnished a firm support for the delicate root. By supporting the top, the cork could easily be washed free from soil by means of a fine spray, after the box was properly tilted. New roots, which were continuously produced, grew along the surface of the cork. They were re-

moved by means of a dissecting scissors or razor blade. One could thus be certain that the seminal root alone was left intact. The first two examinations were made at intervals of 18 days, later ones weekly. Data were obtained from 4 or 5 individuals of each species, but only those of representative plants are presented.

Andropogon furcatus, Sorghastrum nutans, and *Stipa spartea* were grown 77 days, from March 11 until May 27, and *Panicum virgatum* until May 13. *Andropogon furcatus* attained a height of 8.5 inches and developed 11 leaves. All the leaves were green and thriving, except the two lower ones which were reddish and dying. *Sorghastrum nutans* had 6 good leaves

FIG. 3. *Andropogon furcatus* grown on root shown in figure 2. It is 8 inches tall and all but two of the leaves are green and thriving. Note single seminal root and band.

and two dead ones. It was 9 inches tall. The longest of the 8 leaves of *Stipa spartea* extended upward 14 inches. Several of these leaves occurred on the two vigorous tillers. A 63-day-old plant of *Panicum virgatum* had 6 leaves and was 11.5 inches tall.

During the period of growth 20 roots with an aggregate length of 32 inches had been removed from a representative plant of *Andropogon furcatus.* In addition to 29 roots totaling 54 inches in length, several small rhizomes had been excised from *Sorghastrum nutans.* Root production was less in *Stipa spartea* (13 in number and 6 inches in aggregate length) and *Panicum virgatum,* which produced 10 roots averaging an inch in length and also a few rhizomes. In addition to the preceding, a single volunteer plant of *Andropogon scoparius* was grown on its seminal root from June 25 until October 1.

Buchloe dactyloides was grown on the seminal root for two months, June 1 to August 1. The plants tillered profusely, never showed signs of water shortage, and even produced stolons, despite the fact that the diameter of

the single seminal root was only 0.18 mm. (fig. 4). The abundant foliage consisted of 137 leaves varying in length from 0.75 to 3.5 inches, only 14 of which were dead.

Longevity of plants grown on seminal roots

Plants of four species of grasses were grown on the seminal root from June 1 until they succumbed, or until October 1. About two weeks after planting and when the seminal root system was well established, the tops of the plants were fastened to vertical supports and the surface soil carefully washed away so as to expose the upper portion of the roots. Thus, the origin

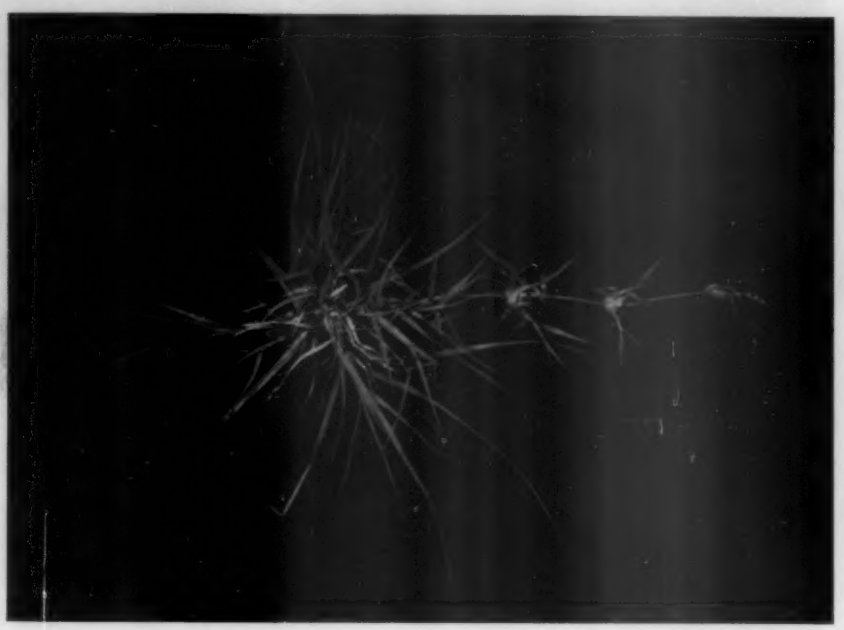

Fig. 4. Single plant of *Buchloe dactyloides* (viewed from above) at the age of 2 months. It was grown on a single seminal root. The stolon is 5 inches long and the leaf area is approximately 20 square inches.

of new roots could be seen. The plants were observed daily and new roots excised as rapidly as they were produced. Conditions favorable to growth were maintained constantly.

These seedlings of *Andropogon furcatus, Sorghastrum nutans, Bouteloua gracilis,* and *Panicum virgatum* had made an excellent growth by July 18. Heights were 10.5, 13, 7, and 10.5 inches, respectively. Among selected representative plants, seven leaves occurred on the parent culm of *Andropogon furcatus* and 3 to 5 on each of the four tillers, a total of 22 leaves. *Sorghastrum nutans* was even better developed. *Bouteloua gracilis* had a total of five tillers and 23 leaves. *Panicum virgatum,* likewise, had tillered freely but most of the foliage (6 leaves) was on the parent culm. A few

plants of each species died late in July and all of *Bouteloua gracilis* had succumbed by August 1. Several plants died during the second week in August when severe conditions promoting high transpiration prevailed. Temperatures of 95° to 100° F. were maintained under a clear sky, relatively low humidity, and high wind. At this time a representative plant of *Andropogon furcatus* had a transpiring area of 12 sq. in. (fig. 5). New roots were initiated in large numbers, but they were removed daily. This continued throughout August but at a decreased rate. Contact with the soil was unnecessary for root production. Such contact promoted the process when the

FIG. 5. Plants developed on the seminal root only when 2 months old on August 1. *Andropogon furcatus* (left) is 11 inches tall; *Sorghastrum nutans* (center) has 5 broad leaves and three leafy tillers; and *Panicum virgatum* (right) is well developed and vigorous.

surface soil was constantly moist and humidity was most favorable for their growth. The base of the plant often became somewhat enlarged as a result of repeated removal of roots. The plants remained alive and mostly green during August. Some produced a few new tillers as the older ones died. Late in August a single plant of *Panicum virgatum* developed a small panicle with four spikelets. The flowers remained in blossom two to three weeks. The plant was 11 inches tall and the largest of its four tillers was 6 inches high and possessed 7 leaves. It obtained all of its water and soil nutrients through a single seminal root only 0.3 mm. in diameter.

In September there was little or no further growth and considerable degeneration occurred. The green color was gradually replaced by one of

reddish brown or yellow as is usual in the fall. Only one or two plants of each species remained alive on October 1, but they were turgid and not wilted.

Experiments with wheat and oats

Seeds of Marquis spring wheat (*Triticum aestivum* L.) were planted on April 8 in boxes 4 feet deep; four plants were grown in each of two boxes.

FIG. 6. Single plant of Marquis spring wheat (left) 66 days old from which the nodal roots had been excised at weekly intervals. (Right) Control and experimental plants of Kherson oats 49 days old. The plant on the right, which is 28 inches tall, had its nodal roots removed weekly.

The seminal roots were banded 18 days later and nodal roots were removed from the plants in one box at weekly intervals until the seminal roots were excavated on June 13. The plants in the other box were grown as controls.

A representative experimental plant was 25 inches tall when in the post-blooming stage on June 13. There were seven tillers, two with spikes in enclosing leaf sheaths (fig. 6). The experimental plants were so similar to

the controls that the only measurable external difference was that they were about two inches shorter. This is remarkable since they had suffered the loss of an average of 51 roots. Some of these were short and unbranched but many were 3 to 5 inches long (the growth of a single week) and well supplied with branches.

An examination showed that all of the main seminal roots and a few of their longer branches had attained depths of 36 to 39 inches and that four of the main seminals had reached the bottom of the box, a depth of 48 inches. This was 10 to 23 inches deeper than seminal roots on the control plants, where an average of 48 roots of the secondary root system also occurred.

Decortication had occurred on the main seminal roots and at least on parts of their large branches to a depth of 20 inches. But the finer branches still appeared fresh and active even in the surface soil, and those of intermediate size below a depth of 6–8 inches. Very similar conditions of moderate deterioration of the upper part of the seminal root system prevailed on the control plants. Below 20 inches depth (14 inches on the controls) the seminal root system was covered with a good cortex and abundant root hairs. The roots appeared fresh and active; in fact, they were much younger than the portions above. Where the seminal root system alone occupied the soil the main roots and their main branches were not only deeper but their laterals branched more profusely, and the branches were longer and more rebranched. Roots were common even in the deepest portion of the soil.

Kherson oats (*Avena sativa* L.) was planted, the seminal roots banded, and nodal roots excised weekly as in the experiment with wheat. When blossoming began on May 27, representative experimental and control plants were 28 and 32 inches tall and possessed 2 and 3 tillers, respectively (fig. 6). Although an average of 21 roots, many of which were 2 to 5 inches long and well branched, had been removed from each experimental plant, there had been no visible wilting at any time. Each plant in both boxes produced but a single panicle.

The seminal roots of plants grown without a secondary root system reached depths of 24 to 26 inches. One of the three seminal roots of such a plant was removed in its entirety from the soil and a diagram made of the position of each main branch. It was then placed in water, the branches properly arranged on black ruled paper, and the entire root drawn to scale without reduction (fig. 7). The presence of an intact cortex with a very large supply of living root hairs below 14 inches where all parts of the root system was apparently functioning was quite significant. The stem just above the seminal roots was 1 mm. in diameter and each of the decorticated seminal roots had a diameter of only 0.2 mm. Branches in the upper 14 inches of soil were mostly decorticated except at their distal ends. But they formed a network, almost cobweb-like in nature, where the minute branches threaded the soil. An abundance of clear, turgid root hairs indicated that they were still active in absorption.

On the controls most of the seminal roots were only 17 to 19 inches deep,

FIG. 7. One of three seminal roots of the plant of Kherson oats shown in figure 6. It is two feet long.

but one extended downward, 34 and another 44 inches. Many of the numerous roots of the secondary system penetrated to the bottom of the box. Decortication of the main seminal roots and their long branches had proceeded farther on the control plants, usually to within a few inches of their growing tips. Compared with the plants with nodal roots removed there were fewer lateral branches, the branches were shorter, and they were not so extensively rebranched. No dense network of branches was found on the controls, although the younger main branches in the deeper soil were abundantly supplied with poorly branched laterals.

Discussion

In all species of perennial grasses studied the seminal root extended 6 to 10 inches in depth during the first 21 days after planting and 7.5 to 27 inches after 41 days. It continued vigorous growth at least until some nodal roots had reached depths of 16 to 24 inches. Depth had increased but little at 59 to 74 days but practically all of the seminal roots were functioning at a time when the abundant tillers were one-half to two-thirds or even fully as tall as the parent culm.

One to 4 nodal roots developed on the parent culm of nearly all of the grasses before tillers appeared. Plants with seminal roots alone not only developed normal, leafy parent culms 8.5 to 14 inches tall but also produced several to many tillers.

Working with *Agropyron cristatum*, *A. pauciflorum*, and *Bromus inermis*, PAVLYCHENKO (**9**) states that the nodal roots supported these perennial grasses within two months after emergence. In the present studies the seminal root systems of all of the grasses were alive at the end of 4 months and at least partly functional. Seminal root systems alone also supported plants of several species for a period of 4 months. OLMSTED (**6, 7**) states that with adequate establishment of adventitious roots of *Bouteloua curtipendula*, the seminal root probably becomes nonfunctional when 6 to 10 weeks old. His plants were grown in two-gallon jars, 9 inches deep. When they were watered at 12- and 20-day intervals, the primary roots remained alive 4 months and in some plants (33 of the 220) it persisted as the only functional part of the root system. In length (7 to 10 inches) and degree of branching in individual plants seminal roots showed a strong inverse correlation with the numbers of functional adventitious roots.

The seminal roots of perennial grasses are not temporary. They remain alive and active 3.5 to 4 months, and possibly under natural field conditions where growth of seedlings is much slower throughout the first season of growth.

The depth of penetration of the seminal root system of several of the smaller cereals has been shown by WEAVER, JEAN, and CRIST (**13**). These roots often penetrated to the greater depth (approximately 6 feet), the nodal roots massing nearer the surface soil. WEAVER, KRAMER, and REED (**14**) found that the seminal roots of winter wheat grew at the average rate of

over half an inch per day during the first 70 days and constituted half of the absorbing area. PAVLYCHENKO (**8**) has traced the development of the seminal root system of wild oats (*Avena fatua*), wheat, rye, and barley, and thus revealed the rapid rate of growth and the total length of the seminal root system. This length ranged from 690 feet in Marquis wheat to 971 in Hannchen barley. He also pointed out the value of the seminal roots in competition with weeds.

HOWARD (**2**) states that on the black soils of India: "The upper soil dries so rapidly after sowing that there is hardly any development of the secondary root system, and practically no tillering. The crop develops slowly on the primary roots." The fact that the weekly removal of the nodal roots of wheat apparently had little effect upon the development of the parent culm is in agreement with the findings of KRASSOVSKY (**4**) who states that the removal of the nodal roots of barley has little influence upon the development and yield of the main stem. She found that "of the plants subjected to a severe wilting at the time of heading, those possessing seminal roots only recovered better than those possessing nodal roots only." OLMSTED (**7**) states that the imposition of a moderate drought probably tends to prolong the life of the primary root system of *Bouteloua curtipendula*. SALLANS (**10**) found that the individual root which contributes most to the final yield of wheat is the primary seminal root, while the first pair of lateral seminal roots is of almost equal importance. KRASSOVSKY (**3, 4**) states that the seminal roots absorb almost double the amount of water per unit dry weight in comparison with the nodal roots. She also states that "the correlation between the time of maximum activity of the seminal roots and the maximum growth of the main stem . . . seems to prove that the seminal roots supply principally the main stem; the nodal roots, the tillers. Removal of the seminal roots in the stage of heading also decreases the yield of the main stem proving that at this stage the barley plant is still dependent on its seminal roots."

Summary

A record was obtained of the development of both roots and tops of 14 species of native and introduced perennial grasses at four stages of growth.

Seminal roots were 6 to 10 inches deep 21 days after planting. There were 1 to 4 nodal roots but rarely a tiller. Twenty days later, when the grasses were mostly 8 to 10 inches tall and well tillered, seminal root depth was 7.5 to 27 inches, and nodal roots were often equally deep.

Seminal roots were usually very fine and the main seminal mostly 0.3 mm. or less in diameter. Branching and length of branches varied greatly among the several species; nearly all were profusely branched. Little or no decortication of the main root had occurred, except in six species where cortex remained only on the distal end. With one exception, cortex of the branches was practically intact.

After 53 to 74 days, when the plants were 5 to 16 inches tall, there were many deeply penetrating nodal roots and numerous tillers one-half to two-

thirds the height of the parent culm. Seminal root depth was 10 to 24 inches. On the smaller branches, and usually on the larger ones, the cortex was intact, but it had sloughed from some of the main seminal roots.

At 90 to 123 days, when the grasses were 9 to 19 inches tall and those of two species had blossomed, the seminal roots, despite 6 to 60 nodal roots, extended deeply and frequently to 24 inches depth. Microscopic examination indicated that, with one exception, about one-third of the seminal root surface appeared normal in one small group, but on the remaining species half to two-thirds of the root system remained intact.

Maximum penetration of seminal roots of 24 to 36 inches was ascertained in certain species. Normally developed plants of several species grew 77 days to heights of 8.5 to 14 inches on the seminal root alone. One plant developed 20 square inches of leaf surface in 60 days on a single, hair-like seminal root. Plants of three species from which all nodal roots were continuously excised tillered freely and remained alive 4 months.

Seminal roots of wheat, with nodal roots excised weekly, were, after the period of blossoming, 3 to 4 feet deep, which was 10 to 23 inches deeper than those of control plants. Similarly, seminal roots of oats usually penetrated deeper than those of the controls. In both species branch roots also penetrated deeper and rebranched more than those of the controls.

Seminal roots of the smaller cereals are not temporary. Those of 14 perennial grasses were usually deep and spread widely. They remained alive and active as absorbing organs 3.5 to 4 months.

UNIVERSITY OF NEBRASKA
LINCOLN, NEBRASKA

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EFFECT OF LIGHT INTENSITY, NITROGEN SUPPLY, AND FRUITING ON CARBOHYDRATE UTILIZATION BY THE COTTON PLANT[1]

FRANK M. EATON AND NEIL E. RIGLER

(WITH EIGHT FIGURES)

The objects of the present investigation were to determine the effects of light intensity and nitrogen supply upon the accumulation of carbohydrates in the cotton plant and in turn upon the utilization of carbohydrates in growth and fruiting activities.

In some measure at least the American upland cotton plant differs from many agricultural plants in that from an early stage in its development, the vegetative and reproductive phases of growth proceed simultaneously; with the production of fruiting branches a non-fruitful condition can exist only as a result of the casting-off of floral buds and bolls during the course of their development. Fruiting branches usually arise from all but the lowest five to eight nodes of the main stalks, of the upland cotton plant, and from each of the nodes of these fruiting branches (fig. 1) there is subtended a floral bud and a leaf; these appear at the same time. The fruiting branches of the cotton plant are sometimes designated as sympodia but in this connection see the discussion of their morphology by COOK (**5**).

The investigation was developed by means of two sand culture experiments. The first was conducted at a relatively low average light intensity of about 900 or 1,000 foot candles at midday and the second under light intensities averaging about 9,000 or 10,000 foot candles at midday. In both experiments four nitrate concentrations were employed—a nitrogen-deficient level, two intermediate levels, and a toxically high level. Each experiment included two series of plants: (1) fruited plants, and (2) plants from which all floral buds were removed before they flowered. The latter plants were included to aid in differentiating between the effects of treatment and the effects of fruiting upon growth and upon carbohydrate accumulation and utilization.

The low-light experiment was conducted under muslin shades in a greenhouse during the winter and the high-light experiment under free exposure outdoors during the summer. Other than for a lowering of the position of the first fruiting branches in medium and late varieties (**23**), but not in early varieties, evidence is lacking of photoperiodism in American upland cottons. More definite effects of photoperiodism in a number of other cottons (**23**) have, however, been recognized. Given adequate light intensities and suitable temperatures, American upland cottons develop fruiting branches and bolls in a characteristic manner during either winter or summer.

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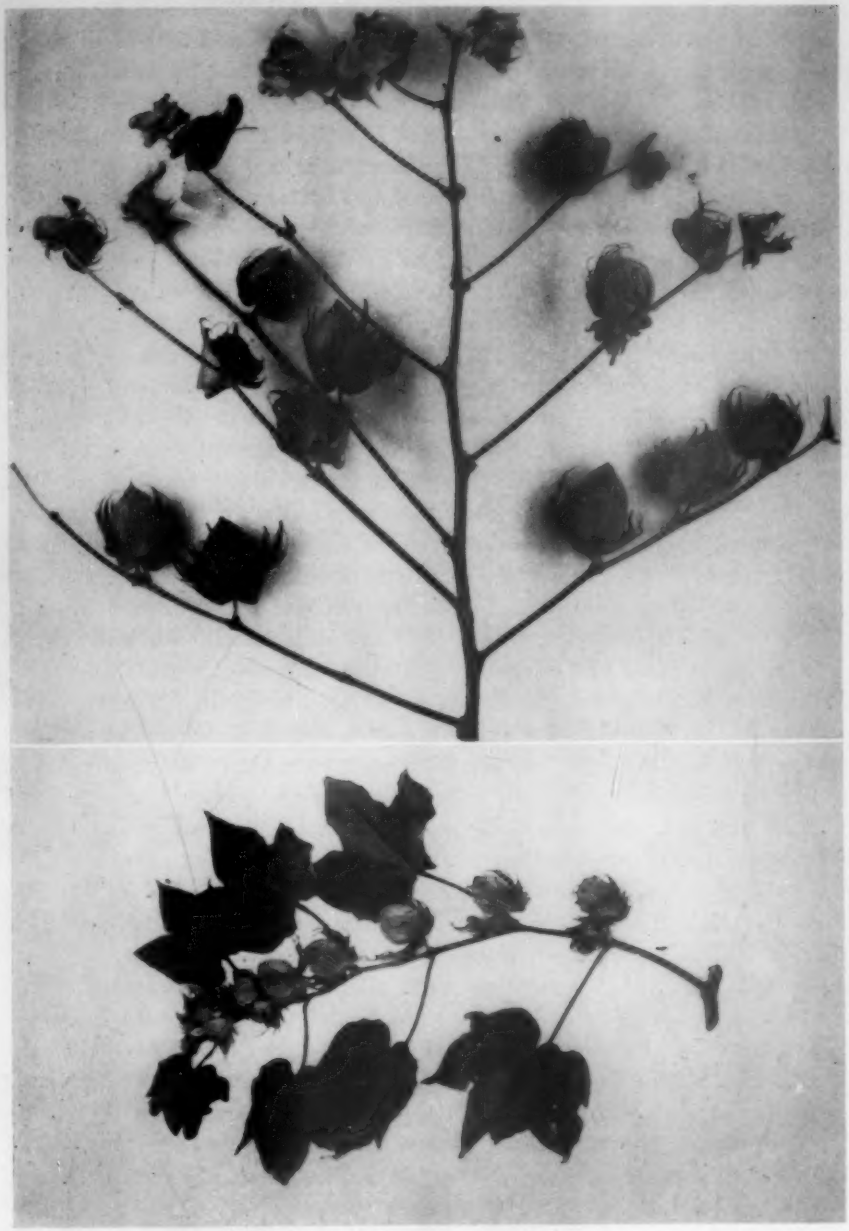

FIG. 1. Above: Upper portion of a cotton plant with leaves removed showing fruiting branches with bolls, vacant nodes (from which floral buds or young bolls had shed) and flowers and floral buds near the ends of the upper branches. Below: A single fruiting branch with a boll, or floral bud, at each node.

Investigations by others have shown that growth, flowering, and cotton production are increased up to certain points by the addition of nitrogen, phosphorus, and potassium, but that the addition of fertilizers has no outstanding effect upon fruitfulness in terms of the percentage of the bolls shed. HARLAND (17) working with Sea Island cotton in the West Indies found this to be the case and concluded that the principal loss in St. Vincent was occasioned by shedding due to fungal and bacterial diseases complicated by heavy rainfall. EWING (15), working with cottons of varied boll sizes, found the mean percentage of bolls shed by three varieties of cotton grown on a fertile valley soil to be 55.3 and on a hill soil 55.5; notwithstanding the production of over three times as many flowers and bolls on the valley soil. In an experiment by NELSON and WARE (30), in which greater growth responses resulted from nitrogen than from phosphate or potassium fertilization, shedding took place under all treatments in proportion to the number of blooms. The percentages of blooms shed were quite uniform in the five-year averages and largely independent of the fertilizer treatments; relatively high shedding percentages were recorded in a dry year. WADLEIGH (34), considering the effects both of nitrogen and of "physiological drought," reported 48.3 and 50.0 per cent. shedding by greenhouse plants supplied with 10 and 100 p.p.m. of nitrogen at low osmotic concentrations, and 46.0 and 58.5 per cent., respectively, at high osmotic concentrations.[2]

In an experiment involving three rates of water application and three of nitrogen that resulted in marked differences in yield, CROWTHER (7), working in the Sudan with Egyptian cotton, found shedding percentages varying between 30.5 and 39.8 for the nine treatments. Between plats in the Sudan the height and yield relationships were so close that the latter could be predicted before picking started. Between years, yields (9) in other experiments, were closely correlated in the Sudan with plant weight, height and the nitrogen content of leaves at various stages of plant development. Correlation failures in the Sudan area are noted in a paper on cotton in Egypt (8) as having been traced to pest damage. In Egypt, the same author (7) found that manuring with nitrogen usually increased plant growth and the number of bolls per plant, and that phosphate increased the amount of seed cotton per boll. The correlations found in the Sudan did not hold well in Egypt (8) because of shedding attributed to environmental factors. Additional data on the effects of nitrogen on the cotton plant, together with references to other literature, have been reported in

[2] Since this manuscript was prepared for publication, WADLEIGH (Arkansas Agr. Exp. Sta. Bul. 446, 1944) has published results on the responses of the cotton plant to variations in nitrogen supply over the non-toxic range; the plants were grown in a greenhouse during the summer. His paper deals extensively with nitrogen utilization and the work is extended to effects on fiber and seed.

Recent data by J. H. TURNER, JR. (Jour. Amer. Soc. Agron. 35: 688–698, 1944) are also pertinent. Under conditions of marked deficiency, the addition of potassium increased leaf retention, boll sizes, and the percentage of flowers that developed into bolls. These effects were most marked in years of high fruitfulness. Small-bolled varieties produced more flowers and shed less, but also yielded less, than did large-bolled varieties.

papers by ARMSTRONG and ALBERT (**2**), BROWN and POPE (**5**), HOLLEY, DULIN, and PICKET (**19**), and DASTUR (**10**).

The literature on the effects of light intensities on cotton is less extensive than that on nitrogen. EWING (**15**) in two seasons found no relation between shedding and cloudy weather but thought that there might have been a relation in a succeeding summer when his light recorder was not in operation. MASON (**27**), working in the West Indies, observed that during the later stages of plant development augmented shedding rates followed dark humid days during which there was generally much rain. He further observed, in keeping with his belief that carbohydrate availability was involved, that the removal of all leaves was followed by the dropping of most of the young fruit. DUNLAP (**11**), in similar connection, found that subjecting plants to light intensities of about 50 f.c. for a number of days caused shedding. Short fruiting branches were obtained by BERKLEY (**3**) in a winter experiment in a Missouri greenhouse under varied lengths of day. Cotton plants grown during the summer by KNIGHT (**22**) under the heavy shade provided by two layers of Hessian cloth had short fruiting branches which produced no flowers; under a single layer of white cloth the plant height was greater, but the numbers of main-stalk nodes and of fruiting nodes were fewer than those of freely exposed control plants and the yield was about half. Data on relations between light quality and fruitfulness are not available, but THARP (**32**) obtained a more rapid growth of young cotton plants in a cellulose-acetate-covered house than in a glass-covered house.

Plan and methods

The four nitrogen concentrations employed in the experiments were 1 (or as noted later, 0.5), 4, 16, and 64 milliequivalents of nitrate per liter of nutrient solution. Twenty-one Stoneville 2B cotton plants, each in a four-gallon jar filled with river sand, were grown at each nitrate level. Of these 21 plants, 14 were allowed to fruit and 7 were debudded. The concentrations in m.e./l. of nitrate and of the other nutrient-solution constituents added to tap water were as follows:

SOLUTION DESIGNATION	Ca	Mg	K	NO_3	SO_4	Cl	HPO_4
1	2	4	3.3	1	4	4	0.3
4	4	4	3.3	4	4	3	0.3
16	16	4	3.3	16	4	3	0.3
64	64	4	3.3	64	4	3	0.3

The tap water contained (m.e./l.) Ca, 0.2; Mg, 0.1; Na, 2.2; HCO_3, 1.6; SO_4, 0.2; and Cl, 0.7. Magnetite was added to the sand as a source of iron at the rate of 0.2 per cent. Boron, manganese, and zinc were added to the nutrient solutions at the rate of 5.0, 0.5, and 0.05 p.p.m., respectively. The concentration of nitrate supplied to the low-nitrate plants in the low-light experiment was reduced from 1.0 to 0.5 m.e./l. during the first week of

flowering. This change was made because up to time of flowering the 1-m.e. plants under low light elongated as rapidly as did the 4-m.e. plants. The solution with 4 m.e. of nitrate was used for all plants prior to the start of differential treatments (see calendar, table I).

The nutrient solutions were added to the sand cultures in units of 2 liters with sufficient frequency to produce copious drainage. It was the general intention that from each two-liter addition there should be between 25 and 50 per cent. of drainage from the jars supporting the larger plants, with a proportionately greater amount of drainage from the jars supporting the smaller plants; the same amount of new solution was supplied to the jars under all treatments. Because of the uptake of nitrogen in excess of water by the plants growing in low-nitrate solutions and of water in excess of nitrate by the plants in the higher nitrate solutions, the nitrate concentrations in the drainage solutions departed substantially from the nitrate concentrations in the solutions applied. It is not implied, therefore, that the concentrations designated for the new solutions were those actually presented to the plant roots. Near the end of the low-light experiment, solutions displaced from representative pots in each treatment, following the addition of 2 liters of new solution after 24 hours' use of the old, had the following nitrate concentrations (m.e./l.):

FRUITED PLANTS				
Nitrate in solution applied	0.5	4.0	16.0	64.0
Nitrate in drainage solution	0.0	8.0	36.8	94.6
Percentage of solution recovered	65.0	28.0	37.0	64.0
DEBUDDED PLANTS				
Nitrate in solution applied	0.5	4.0	16.0	64.0
Nitrate in drainage solution	0.0	9.1	37.2	101.7
Percentage of solution recovered	57.0	39.0	30.0	36.0

The low-light intensities during the winter in the greenhouse experiment were obtained by the use of muslin shades suspended from rings strung on overhead wires. The shades were used on bright days, but on cloudy days they were drawn back. On cloudy days, without the shades, the daily light intensities at the tops of the plants customarily varied between 300 and 800 f.c. at midday. On bright days under the shades the light intensity at different positions in the greenhouse varied between 700 and 1,500 f.c.; this variation on bright days was caused principally by the partial shadow cast on one of the benches by overhead ventilators. The variation between the benches was equalized to some extent by shifting the pots so that each occupied 3 different positions during the flowering period; one shift in the position of the pots was made prior to flowering. The high-light experiment was conducted outdoors during the summer under free exposure. The outdoor light intensity on a horizontal plane varied from about 9,000 to 11,000 f.c. on bright days at midday. On cloudy days, of which there were relatively few during the fruiting period, the midday intensity probably did not fall below 3,000 f.c. A value between 900 and 1,000 f.c. during the low-

light experiment and between 9,000 and 10,000 f.c. during the high-light experiment would probably approach with sufficient accuracy the average midday light intensities during the respective flowering periods. A Weston photometer was used for the light intensity measurements.

Recognizing that plant development and boll development would be slower during short days in the greenhouse than outdoors in the summer, an effort was made to compensate roughly for the difference in the number of hours per day available for photosynthesis by extending the duration of the winter experiment (table I). The ratio of day lengths during the fruit-

TABLE I

CALENDARS FOR THE LOW-LIGHT AND HIGH-LIGHT EXPERIMENTS

Period or treatment	Low light				High light			
	Date	Day length	Elapsed time	Interval	Date	Day length	Elapsed time	Interval
		hours	*days*	*days*		*hours*	*days*	*days*
Planted	Oct. 20	11.3	.....	.....	Apr. 8	12.7	.....	.....
Diff. nitrate treatments started*	Nov. 18	10.5	29	29	May 6†	13.5	28	28
First flowers	Dec. 27	10.1	68	37	May 30	14.0	52	24
Cropped	Feb. 16	11.1	119	51	July 9	14.1	92	40

Cropping procedure: 10 A.M. C.S.T.‡ Duplicate samples of sound mainstalk leaf blades were collected from 12th to 16th nodes of each plant.

11 A.M. to 1 P.M. Cut and weighed bolls and tops of plants.

1 P.M.§ The roots of all plants were pulled out, washed free from sand, and the root bark was then separated for duplicate samples.

* Started using shades to reduce light intensity in low-light experiment on December 1.

† Plants removed from greenhouse to free exposure outdoors.

‡ At 10: 30 A.M. the day had advanced about 34 per cent. beyond sunrise on February 16 and 38 per cent. beyond on July 9.

§ At 1: 30 P.M. the day had advanced about 61 per cent. beyond sunrise on February 16 and 60 per cent. beyond on July 9.

ing periods in the two experiments was (winter/summer) $10.6/14.1 = 0.75$; the ratio of the periods allowed for fruiting was (summer/winter) $40/51 = 0.78$; and the ratio of the observed lengths of the periods from planting to first flowers was (winter/summer) $52/68 = 0.76$. The mean temperatures during the fruiting periods were similar; in the summer experiment the mean maximum was 90.7°, the mean minimum 71.9°, and the average mean daily temperature 81.3° F. The greenhouse thermostat in the winter experiment was set at 80° and there were substantial plus and minus departures from this that varied with the exterior temperature and radiation.

The time of cropping in the respective experiments was selected to coincide as nearly as possible with the probable approaching end of the period of weight increase of the first bolls set. As shown in figure 2, the daily rate of dry-weight increase of a cotton boll, although not linear, is substantial

from about the fifth day after flowering to about ten days prior to opening. Inasmuch as boll development was slower in the low-light greenhouse experiment than in the high-light experiment, the same average rate of carbohydrate movement to each boll per day did not occur under the two sets of conditions. With this qualification in mind, the number of developing bolls per unit of plant weight has certain advantages in a nutritional interpretation over shedding percentages which take as their basis the total number of buds or the number of flowers. All of the bolls of both experiments were still green when the plants were cropped and no data on the mature bolls were secured.

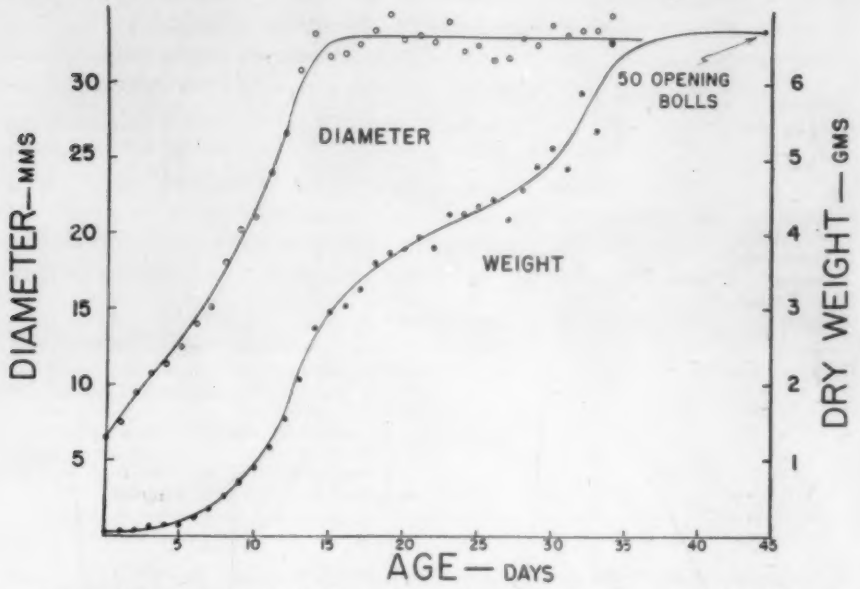

FIG. 2. The diameters and dry weights of Acala cotton bolls by days from flowering to maturity at Sacaton, Arizona, in 1926. The final 50 bolls (ages 42 to 46 days) were removed from the plants on September 20 and the others (4 to 20 bolls for each mean) were removed on August 26.

The fresh-leaf and root-bark samples were placed in, and the sugars extracted with, hot 80 per cent. alcohol (including water of the samples); neutral lead acetate and sodium oxalate were used in clarifying and deleading. Analyses for sucrose were made on solutions which had been inverted with hydrochloric acid for 24 hours at room temperatures. After gelatinizing 2-gram samples of the residues from the alcohol extraction, starch was determined by treating with 20 ml. of a 0.5 per cent. solution of undiluted diastase (supplied by courtesy of Parke, Davis and Company) for one hour at 55° C., again heating to boiling, cooling to 55° C., and re-treating with diastase, as before, for one hour or longer if the material still showed color with iodine solution. After cooling, the supernatant liquid was centrifuged from the residue and the latter washed three times. The solution was then

made to volume and hydrolyzed with acid for dextrose determination. The residue after the hydrolysis and extraction of starch was refluxed with 2 per cent. hydrochloric acid for 2.5 hours and the sugars reported as hemicellulose. The sugar determinations were made according to the method

TABLE II

GROWTH AND FRUITING OF COTTON PLANTS AS INFLUENCED BY LIGHT INTENSITY AND BY NITRATE SUPPLY. ALL VALUES ARE AVERAGES PER PLANT

TREATMENT AND NITRATE SUPPLY	HEIGHT	MAIN-STALK NODES	FRUITING BRANCH NODES*		FRESH WEIGHTS			FLOWERS		DEVELOPING BOLLS	
			TOTAL	ON CENTRAL BRANCHES†	STEMS AND LEAVES	BOLLS	TAP ROOTS	TOTAL	SHED	TOTAL	PER 100 GRAMS OF STEMS AND LEAVES
m.e./l.	*cm.*	*no.*	*no.*	*no.*	*gm.*	*gm.*	*gm.*	*no.*	*%*	*no.*	*no.*
LOW LIGHT											
Fruited											
1 (0.5)	70	18.7	.....	1.6	128	117	11.8	6.9‡	17‡	5.7	4.46
4	91	23.7	.....	2.6	319	153	22.2	12.5‡	9‡	11.4	3.47
16	95	23.9	.....	2.6	328	150	21.6	12.5‡	10‡	11.2	3.36
64	79	22.1	.....	2.6	239	142	14.6	10.9‡	12‡	9.6	4.09
Debudded											
1 (0.5)	123	23.6	25	2.7	197	.....	17.6	.....	.....	.....	.....
4	121	25.3	42	4.3	417	.....	30.1	.....	.....	.....	.....
16	116	26.0	46	4.2	443	.....	29.2	.....	.....	.....	.....
64	113	25.5	43	4.1	350	.....	18.2	.....	.....	.....	.....
HIGH LIGHT											
Fruited											
1	60	17.4	14	1.6	107	170	10.4	13.1	46	7.2	6.75
4	91	21.9	52	5.3	382	458	28.1	33.7	35	22.9	6.42
16	92	21.4	58	4.6	367	468	29.1	33.7	35	22.9	6.43
64	81	20.0	38	4.0	252	370	22.0	32.3	43	18.4	7.58
Debudded											
1	86	19.9	48	3.7	158	.....	20.9	.....	.....	.....	.....
4	113	23.4	107	4.5	555	.....	46.6	.....	.....	.....	.....
16	108	23.4	118	5.3	566	.....	53.8	.....	.....	.....	.....
64	102	21.9	104	5.4	445	.....	41.8	.....	.....	.....	.....

* Fruiting-branch nodes include all of those producing flowers or squares with a bract width of 15 mm. or more. There was a heavy shedding of very small squares by the fruited plants under low light but square shedding by the debudded plants under low light and by all plants under high light was negligible. Nodes counted on the fruited plants under low light were based on the size of the subtended leaves.

† Fruiting branches subtended by the 10th, 12th, and 14th main-stalk nodes.

‡ These values are so largely influenced by the heavy shedding of floral buds (see note above) that they have little significance.

of WILDMAN and HANSEN (35) except that in titration, potassium permanganate and ortho-phenanthroline indicator were used. By the foregoing procedure the division between "starch" and "hemicellulose" is drawn on the basis of diastatic activity.

It is well known that diastase is generic in its action since it includes

a number of enzymes which are capable of attacking starch, dextrin, and like substances. To add undue specificity to "starch" values in plant nutrition and thereby exclude from consideration closely related carbohydrates

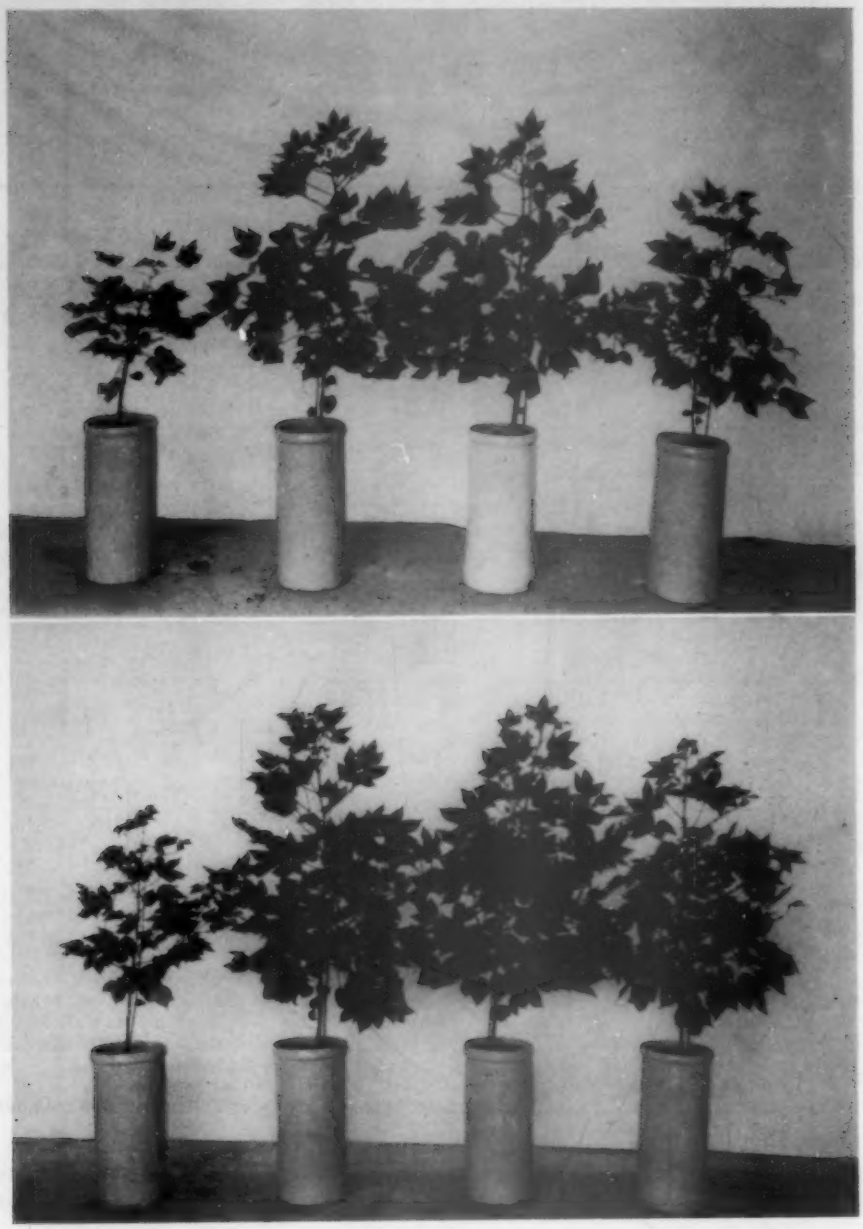

FIG. 3. Fruited (above) and debudded (below) cotton plants of average height at the end of the high-light experiment. Left to right: 1, 4, 16, and 64 m.e./l. of nitrate in nutrient solution.

is often undesirable and it is also undesirable to include these other compounds as "hemicellulose" since in such event the significance of the latter fraction is reduced.

Experimental procedure

Plant development and fruiting

In both the high- and the low-light intensity experiments the vegetative development of the cotton plants (fig. 3) and the number of bolls they produced were reduced both by the low and by the high concentrations of nitrate (table II). In terms of relative fruitfulness, however, the plants on the solutions of very low and very high nitrate were slightly more productive than the plants growing on the substrates with intermediate nitrate;

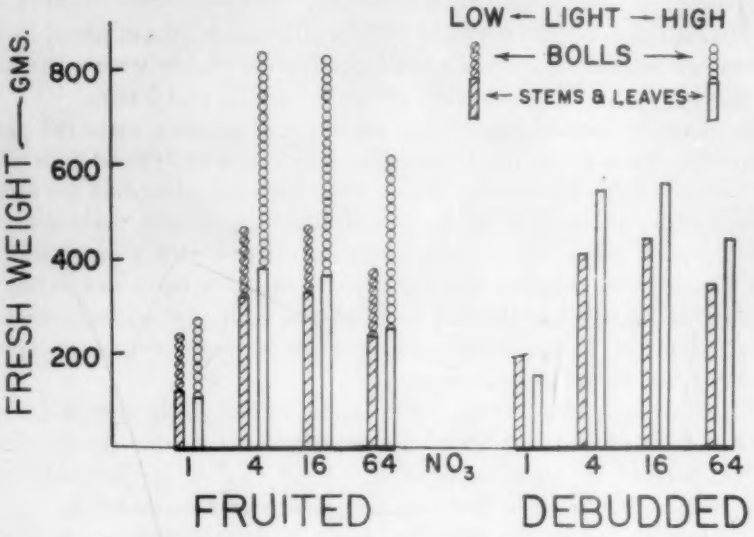

Fig. 4. Fresh weights of stems and leaves, and of bolls in the low-light and high-light experiments.

i.e., they produced more bolls per 100 grams of fresh weight of stems and leaves. These differences in relative fruitfulness were statistically significant in the low-light but not in the high-light experiment.

Under the high-light intensity the plants were markedly more fruitful than under low light, but high light did not result in a corresponding increase in the weight of stems and leaves (fig. 4). The average number of bolls per plant under low light (all nitrate levels) was 9.2 and under high light 17.9, whereas the average fresh weight of stems and leaves under low light was 253 grams and under high light 277 grams. Low light thus curtailed fruiting to a far greater extent than it did vegetative development. The fresh weight of bolls averaged 51 per cent. of the fresh weight of stems and leaves under low light and 132 per cent. under high light.

The heights of the plants and the number of main-stalk nodes were not

affected very much by light intensities, but light had a notable effect upon the number of nodes developed by the fruiting branches. Of the debudded plants those under high light developed twice as many fruiting-branch nodes as did those under low light. Similarly, except at the low nitrogen level, the fruited plants under the high light had many more nodes on their central fruiting branches than did those under low light. It appears accordingly that low-light intensity (or possibly the reduced carbohydrates associated with the low light) has effects on the development of fruiting branches that are distinct from its effects on the development of the main stalk.

There was a negligible amount of shedding of floral buds by both the fruited and the debudded plants under high light, but the shedding of floral buds was a prominent feature in the reactions of the fruited plants, but not of the debudded plants, under low light. The buds were removed from the debudded plants when they had involucral bract widths of about 15 mm., whereas the buds shed from the low-light fruited plants were customarily very small; usually they had bract widths between 2 and 5 mm.

The effects of nitrate supply and the effect of fruiting upon the growth of the cotton plant are shown by the diagrams in figure 5; these data are all from the high-light experiment. Favorable nitrate supplies, like the removal of floral buds, resulted in an increased number of both main-stalk and fruiting-branch nodes. Only nodes bearing squares with a bract width of about 15 mm. were counted and for this reason no squares are shown terminating the branches of the debudded plants. All of the higher branches of the debudded plants were actively growing and producing squares, however, at the end of the experiment.

The diagrams (fig. 5) bring out a salient difference in the effects upon growth of the deficient and toxic concentrations of nitrate. At the time of cropping none of the terminal buds of the main stalks of the low-nitrogen plants were active and few fruiting branches were terminated by squares. The high-nitrate plants, on the other hand, were still growing at the end of the experiment but at a slower rate than the plants in the intermediate concentrations; all of the terminal buds of the high-nitrate plants were active at this time and nearly all of the upper branches were still forming leaves and squares. Nitrogen deficiency, in other words, prevented vegetative expansion as these plants set the last of their bolls, whereas an excess of nitrate only slowed development.

High nitrate reduced the fresh weight of both fruited and debudded plants, under both light intensities, out of proportion to its effect on the number of main-stalk and fruiting-branch nodes; the morphological differentiation of parts by the high-nitrate plants was thus more rapid than was growth as measured either by internode length or weight of plant. In high nitrate, as in low nitrate, it was not only plant height that was reduced, but also the diameters of the main stalks and branches and the area of the leaves. CROWTHER (7) has dealt with the effects of nitrogen and of water on growth and differentiation in cotton over the non-toxic ranges of nitrogen

supply. He concluded that differences in internode lengths, which are correlated with yield, imply critical variation in water supply, whereas node numbers depend upon the amount of nitrogen. Data presented in

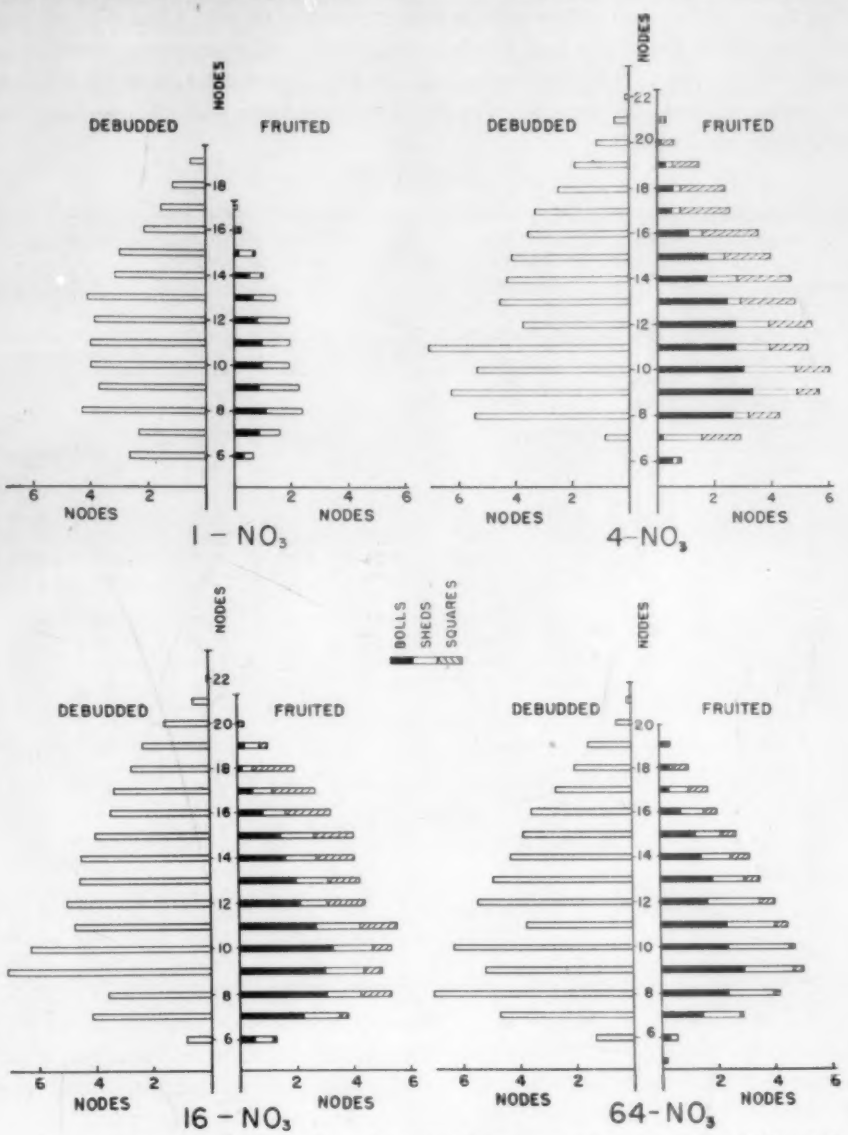

FIG. 5. Number of main-stalk and fruiting-branch nodes and number of bolls, sheds, and squares (floral buds) per fruiting branch at the four nitrate levels in the high-light experiment. See illustrations of the fruiting branches of cotton, fig. 1.

another paper (**13**) have shown that water movement to cotton leaves is probably not impaired by saline substrates, but that with saline substrates the water in the xylem vessels is continuously under tension.

The high-nitrate concentration was more toxic to the fruited than to the debudded plants. Under low light the weight of stems and leaves of the fruited plants supplied with 64 m.e./l. of nitrate was 28 per cent. below the mean of the two intermediate concentrations (4 and 16 m.e.), whereas the weight of the stems and leaves of the debudded plants was reduced by only 19 per cent. The corresponding growth depressions in the high-light experiment were 33 per cent. for the fruited plants and 21 per cent. for the debudded plants.

TABLE III

CARBOHYDRATE AND NITROGEN ACCUMULATION IN LEAVES OF FRUITED AND DEBUDDED COTTON PLANTS AT FOUR NITROGEN LEVELS AND UNDER LOW- AND HIGH-LIGHT INTENSITIES. ALL VALUES AS PERCENTAGE OF FRESH WEIGHT

TREATMENT AND NITRATE IN CULTURE SOLUTION	MOISTURE	CARBOHYDRATES					NITROGEN	
		REDUCING SUGARS	SUCROSE	STARCH	SUM	HEMICELLULOSE	NO_3	TOTAL
m.e./l.	%	%	%	%	%	%	%	%
LOW LIGHT								
Fruited								
1 (or 0.5)	.....	0.08	0.21	0.26	0.53	1.28	0.006	0.44
4	.....	0.03	0.17	0.23	0.43	1.10	0.078	0.48
16	.....	0.04	0.18	0.24	0.46	1.04	0.091	0.53
64	.....	0.06	0.21	0.25	0.52	1.08	0.099	0.63
Debudded								
1 (0.5)	.....	0.10	0.23	0.41	0.74	1.52	0.004	0.45
4	.....	0.05	0.18	0.24	0.47	1.17	0.078	0.50
16	.....	0.09	0.18	0.26	0.53	1.24	0.096	0.55
64	.....	0.08	0.20	0.26	0.54	1.19	0.108	0.67
HIGH LIGHT								
Fruited								
1	78.0	0.15	0.26	1.21	1.62	2.77	.....	0.57
4	76.5	0.13	0.28	1.34	1.75	2.65	.....	0.64
16	75.2	0.13	0.33	1.59	2.05	2.83	.....	0.65
64	72.6	0.18	0.31	1.78	2.27	2.83	.....	0.75
Debudded								
1	76.4	0.20	0.25	1.79	2.24	3.39	.....	0.51
4	76.1	0.14	0.34	1.54	2.04	2.59	.....	0.69
16	73.4	0.15	0.43	2.04	2.62	3.01	.....	0.76
64	70.9	0.16	0.37	2.82	3.35	4.00	.....	0.84

In both of the experiments all vegetative branches were removed just before the appearance of the first flowers, but vegetative branches which appeared later were not disturbed. The appearance and growth of new vegetative branches became a prominent feature of the subsequent growth of the high-light debudded plants but in the other three treatments their growth was negligible. The debudded plants supplied with 1, 4, 16, and 64 m.e. of nitrate in the high-light experiment developed, respectively, an average of 1.6, 9.4, 10.4, and 8.0 vegetative branches per plant from their main-stalk nodes and 0, 14.1, 13.8, and 10.6 vegetative branches from fruiting-branch nodes. The counts of vegetative branches included those that had two or more leaves.

Carbohydrate and nitrogen accumulation

A factorial summary of the carbohydrate and nitrogen accumulation data is presented in figure 6 and the detailed effects of the individual treatments are presented in tables III and IV. In the factorial summary the bars corresponding to each level of nitrate supply are the means of the 8 values representing the concentrations found in the leaf and root tissues of the fruited and debudded plants at low- and high-light intensities. Simi-

TABLE IV

Carbohydrate and nitrogen accumulation in root bark of fruited and debudded cotton plants at four nitrogen levels and under low- and high-light intensities. All values as percentage of fresh weight

Treatment and nitrate in culture solution	Moisture	Carbohydrates					Nitrogen	
		Reducing sugars	Sucrose	Starch	Sum	Hemicellulose	NO_3	Total
	Low light							
m.e./l.	%	%	%	%	%	%	%	%
Fruited								
1 (0.5)	.....	0.23	0.41	0.54	1.18	2.24	0.028	0.11
4	.....	0.20	0.58	0.38	1.16	2.21	0.090	0.18
16	.....	0.21	0.63	0.37	1.21	2.06	0.117	0.21
64	.....	0.21	0.57	0.32	1.10	2.22	0.128	0.33
Debudded								
1 (0.5)	.....	0.42	1.62	2.01	4.05	2.61	0.006	0.14
4	.....	0.31	1.32	0.52	2.15	2.32	0.073	0.20
16	.....	0.41	1.46	0.50	2.37	2.23	0.102	0.25
64	.....	0.34	1.30	0.56	2.20	2.24	0.117	0.43
	High light							
Fruited								
1	80.8	0.13	0.25	1.51	1.89	2.59	.....	0.13
4	82.5	0.27	0.44	1.09	1.80	2.79	.....	0.16
16	79.4	0.48	1.06	1.37	2.91	3.19	.....	0.20
64	77.9	0.67	1.07	1.65	3.39	3.12	.....	0.23
Debudded								
1	77.6	0.82	2.43	2.77	6.02	2.96	.....	0.13
4	77.9	0.71	1.89	1.81	4.41	3.19	.....	0.27
16	73.8	0.68	2.28	3.90	6.86	3.48	.....	0.35
64	70.9	0.70	2.16	5.61	8.47	3.94	.....	0.45

larly, the overall effect of fruiting on accumulation of carbohydrate and nitrogen is represented by the respective means of the 16 values for the concentrations found in the leaves and root bark at the four nitrate levels under low and high light. The effects of light intensity and of tissue sampled (leaves and root bark) are likewise shown as the mean of sixteen values. The factorial summaries serve the worthwhile purpose of orientation as regards trends under varied conditions but a consideration of the detailed results remains necessary for effects of interactions.

Sugars and starch.—On the basis of the factorial summary (fig. 6), deficient nitrogen resulted in an added accumulation of reducing sugars,

sucrose, and starch. By reference to tables III and IV, however, it is to be observed that this effect, although pronounced in the debudded plants, was minor or in some instances reversed in the fruited plants.

With increasing concentrations of nitrate above 4 m.e./l. successive increases are shown in the factorial summary for the concentration of reducing sugars, sucrose, and starch. This effect was minor, or in some instances reversed, in both the fruited and debudded plants under low light; it usually occurred in the fruited plants under high light, and was consistent

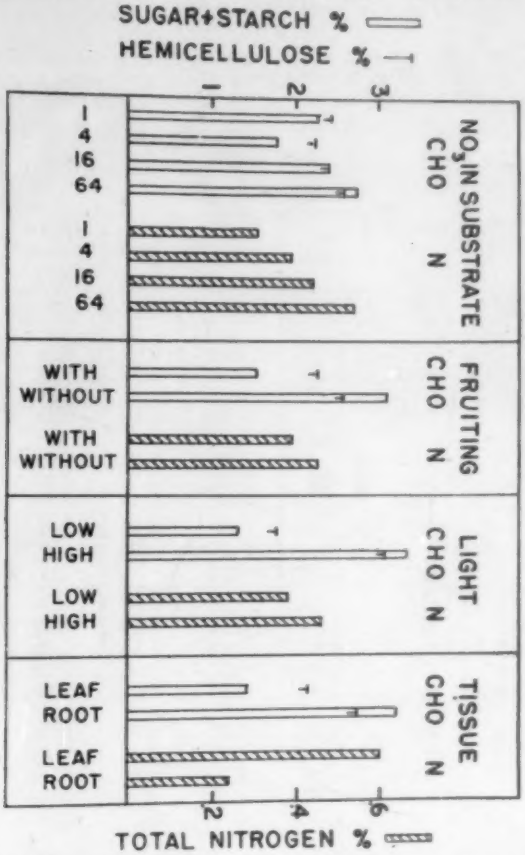

FIG. 6. Factorial summary of effects of nitrate supply, fruiting, light, and tissue sampled on the accumulation of sugars plus starch, hemicellulose, and nitrogen.

except for sucrose in the debudded plants under high light. The effect of high nitrate on sucrose is at variance with its effect on reducing sugars and starch; sucrose tended to decrease rather than to increase in the 64 m.e./l. concentration relative to 16 m.e./l.

Debudding resulted in greater increases in carbohydrates in the root bark than in the leaves. The small additional accumulation of carbohydrates that is observable in the leaves of the debudded, as compared with the

fruited plants, probably reflects the utilization of carbohydrates by the bolls; the utilization of carbohydrates by the bolls would reduce carbohydrate in the phloem and thereby possibly promote a more rapid movement out of the leaves. The most outstanding effect of debudding was the increase in sucrose in the root bark. This increase in sucrose concentration in the root bark accords well with the conclusion of MASON and MASKELL (**28**) that carbohydrate movement into the cotton boll occurs primarily as sucrose. In the present experiments, debudding resulted in the sucrose that otherwise would have gone into the bolls moving on to the root bark and the increase in sucrose in the root bark of the debudded plants exceeded the increases in reducing sugars and starch.

HEMICELLULOSE

In the leaves, figure 7, hemicellulose tended to change as starch changed and its concentration exceeded that of starch. High light caused a large increase in both fractions and in the debudded plants both of these fractions were markedly influenced by nitrate supply.

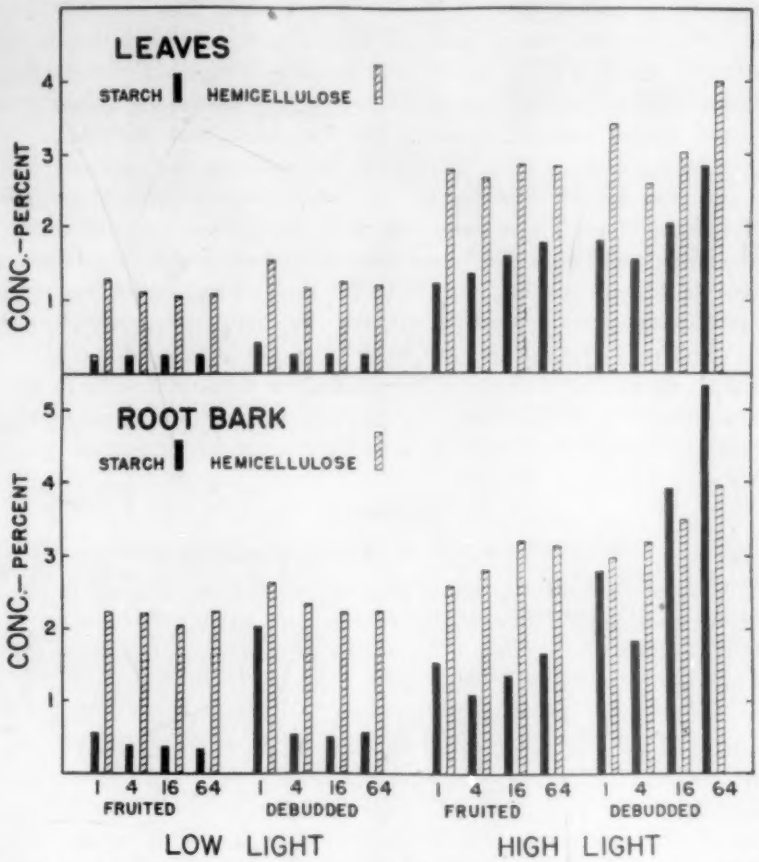

FIG. 7. Accumulation of starch and hemicellulose in leaves and root bark.

In the root bark, starch was increased when nitrogen was deficient whereas hemicellulose showed little variation.

Debudding resulted in large increases in the concentrations of starch found in the root bark but had only a minor effect on the concentrations of hemicellulose. This is difficult to understand for the reason that a substantial movement of the sugars responsible for hemicellulose formation presumably takes place to the fruiting structures.

Nitrogen

A general correlation extends throughout the data between the concentration of nitrate supplied in the substrate and the concentrations of nitrogen found in the leaves and in the root bark. The 1, 4, 16, and 64 m.e./l. concentrations of nitrate (in the solutions as applied) resulted respectively in average concentrations of 0.49, 0.58, 0.62, and 0.72 per cent. total nitrogen in the leaves and 0.13, 0.20, 0.25, and 0.36 per cent. total nitrogen in the root bark. Each four-fold increase in the concentration of the nitrate in the solution applied resulted in small increases within the plant and these successive increases in the plants were of similar magnitude. The entire 64-fold increase in the nitrate concentration of the culture solution failed to double the nitrogen content of the leaves or to quadruple root bark concentrations.

Both the debudding and the use of high light were accompanied by added sugar and starch accumulations in the root bark, and with these added carbohydrate accumulations, there were increases in the concentrations of nitrogen. This result is in accord with other results (**14**) wherein direct correlations were shown between sugars in the fibrous roots of cotton and the accumulation of bromine, potassium, and nitrogen. In that work, as in the present, there is a substantial basis for the conclusion that the extent of nitrogen uptake is influenced by and may be directly dependent upon the extent to which carbohydrates are moved into the fibrous roots.

Nitrate accumulation within the plants was measured only in the low-light experiment. In the leaves the ratios of nitrate to total nitrogen averaged about 1 to 6, but in the root bark the proportion of nitrate was much greater.

Discussion

Carbohydrate accumulation and utilization

Substantial uncertainty must always accompany any attempt to interpret the significance of data on carbohydrate accumulation. The concentrations found within a tissue at time of sampling represents at best only a fluctuating balance between the rate of input, whether from photosynthesis or by translocation from other tissues, and the rates of export and utilization; the latter may be by respiration or by the elaboration of the carbohydrates into organic acids, nitrogenous compounds, cellulose, etc. The tissues selected for sampling may or may not be representative of the plant as a whole. Assuming that the tissues are representative, however, a greater accumulation in one set of plants than in another can mean either that the treat-

ment imposed favored a higher rate of photosynthesis or that some constituent or condition limited utilization. This, as is well known, is a way of saying that the extent of accumulation finds significance largely in the light of the inferences that are drawn with respect to the probable effects of the variables under investigation. If these inferences fit the findings, or the departures can be explained, the investigator is led to believe that some advance has been made toward an understanding of causes and effects.

TABLE V

RECAPITULATION OF VEGETATIVE GROWTH AND FRUITING, AND OF CARBOHYDRATE AND TOTAL NITROGEN ACCUMULATION

NITRATE IN NUTRIENT SOLUTION	FRESH WEIGHT		BOLLS		AVERAGES OF LEAVES AND ROOT BARK		
	STEMS AND LEAVES	BOLLS	PER PLANT	PER 100 GRAMS STEMS AND LEAVES	SUGARS PLUS STARCH	TOTAL NITROGEN	CARBOHYDRATE/NITROGEN RATIO
	m.e./l.	*gm.*	*gm.*	*no.*	*no.*	%	%	
LOW LIGHT							
Fruited							
1 (0.5)	128	117	5.7	4.5	0.85	0.29	2.93
4	319	153	11.4	3.8	0.80	0.33	2.42
16	328	150	11.2	3.4	0.84	0.37	2.27
64	239	142	9.6	4.1	0.81	0.48	1.69
Debudded							
1 (0.5)	197	.....	.....	.....	2.40	0.30	8.00
4	417	.....	.....	.....	1.31	0.35	3.74
16	443	.....	.....	.....	1.45	0.40	3.62
64	350	.....	.....	.....	1.37	0.55	2.49
HIGH LIGHT							
Fruited							
1	107	170	7.2	6.8	1.76	0.35	3.03
4	382	458	22.9	6.4	1.78	0.40	4.45
16	367	468	22.9	6.4	2.48	0.43	5.76
64	252	370	18.4	7.6	2.83	0.49	5.77
Debudded							
1	158	.....	.....	.....	4.13	0.51	8.10
4	555	.....	.....	.....	3.23	0.69	4.68
16	566	.....	.....	.....	4.74	0.76	6.23
64	445	.....	.....	.....	5.91	0.84	7.03

In the present work a greater accumulation of sugars and of the compounds grouped as starch was found to result from the use of high-light intensities than low-light intensities and further accumulation resulted when the plants were debudded. A somewhat greater accumulation was found also in the plants on nutrient solutions that were deficient in nitrate and again in those that were supplied with an excess of nitrate than in those supplied with the intermediate concentrations. The tendency toward an accumulation of carbohydrate with deficient nitrogen has long been recognized (**24**) and the accumulation with an excess of nitrate may be similar in cause with the accumulation found to be associated with an excess of

chloride and sulfate salts (**16**). In both instances, table V, such accumulation of carbohydrate was associated with depressed growth; i.e., the responsible factors depressed utilization more than they did photosynthesis.

From the standpoint of the main interests that prompted the present investigation the effects of nitrogen supply has proven to be of lesser significance than the effects of light and of debudding. This is the case for the reason that the data have reaffirmed, in accord with the evidence from other sources, that variation in nitrogen supply, in conjunction with abundant supplies of other nutritional elements, does not greatly alter the relative fruitfulness of the cotton plant. Over the range of 1 to 64 m.e. of nitrate, the partition in carbohydrate utilization between vegetative development and fruiting remained essentially unchanged; per unit of fresh weight of leaves and stems the number of bolls produced was not affected to any outstanding degree. This lack of any pronounced effect of nitrogen on relative fruitfulness was found to be the case both under the diverse conditions represented by light intensities of 1,000 f.c. associated with short winter days in a greenhouse and by those represented by intensities of 10,000 f.c. in midsummer out-of-doors.

Turning to the effect of light, it seems evident (table V) that the midday light intensities of about 1,000 f.c. were insufficient for the manufacture of enough carbohydrate to satisfy the growth capacities of the plants. Recalling that the duration of the low-light experiment was extended roughly in proportion to the reduction in day length, it was found at time of cropping that the weights of stems and leaves produced under low light were essentially equal to the weights of stems and leaves under high light. This was not the case, however, with weights of bolls. The weight of bolls produced by the high-light plants was 2.5 times as great as the weight produced by the low-light plants. In other words, the entire growth curtailment under low light was represented by the curtailment of fruiting activities. Or, conversely, the extra photosynthetic product produced under high light was utilized principally in boll production.

The average carbohydrate concentrations (sugar plus starch) were 2.7 times greater (average of leaves and root bark at all nitrate levels) under high light than under low light, and high light increased the weight of bolls by 2.5 times. The close similarity in these values is of course coincidental, but to follow an interpretation that might be placed on some of the research by MASON, MASKELL, and PHILLIS (**26, 28, 29**) the increased carbohydrate concentrations within the high-light plants could be construed as having brought about an enhanced rate of movement of carbohydrates, along descending sucrose gradients, into the reproductive structures. This explanation, however, does not seem to be wholly adequate, especially when it is remembered that the potentials for movement into the vegetative buds and roots were similarly increased.

Debudding under both low and high light resulted in marked increases in vegetative development and also increased carbohydrate concentrations.

In no instance, however, did the stems and leaves of the debudded plants weigh as much as the stems and leaves plus the bolls of the fruited plants; notwithstanding the greater leaf area for photosynthetic activity of the former. In other words the *increase* in carbohydrate utilization in the vegetative parts of the debudded plants did not compensate for the utilization that took place in the bolls of the fruited plants. The fact, nevertheless, that vegetative growth was increased by bud removal leaves no doubt that the growing bolls did curtail vegetative expansion. It is probable that the carbohydrate utilization by the bolls was an important, if not the only cause, of the lesser vegetative growth of the fruited plants.

Under low light the weight of the vegetative growth of the fruited plants exceeded the weight of bolls whereas under high light this situation was reversed. The utilization partition between vegetative and reproductive activities was thus in favor of the reproductive under high light and in favor of the vegetative under low light. Or, stated otherwise, some part of the dominance of reproductive activities that existed under high light was lost under low light. Additional consideration will be given to the light factor after a further consideration of carbohydrate utilization by the bolls.

Effect of light on carbohydrate utilization by bolls

The idea has been prevalent in the literature for many years that the number of bolls retained by a cotton plant is a function of the number it can "nourish" or "support." The availability of water and of the essential elements being assumed ample, the carbohydrate supply has customarily been regarded as a chief limiting factor in boll production. The present data afford an opportunity, possibly an especially favorable one, for further examining this hypothesis. As a means of simplifying the discussion, attention will be restricted to the plants supplied with 4 and 16 m.e. of nitrate under low and high light. The growth and sugar and starch data from these plants have been brought together in table VI.

As a first consideration, it may be observed, table VI, that under low light the fruited 4- and 16-m.e. plants had about the same fresh weights, set about the same number of bolls, and had essentially equal concentrations of sugars and starch in their leaves; the concentrations in the root bark were higher than in the leaves, but between the 4- and 16-m.e. treatments there is no indication of a significant difference in any of these measurements. It would be reasonable to conclude that the carbohydrate supply limited the fruiting of these low-light plants and that it also limited their vegetative growth; such a conclusion is supported by the fact that the debudded plants had substantially higher concentrations of carbohydrates in their root barks than did the fruited plants and that they made substantially more vegetative growth and produced many more floral buds.

Similarly, under high light, and again comparing the 4- and 16-m.e. fruited plants, there was little difference between these treatments in fresh weight, or in the number of bolls that were set; both groups of plants shed

TABLE VI

RECAPITULATION OF GROWTH AND CARBOHYDRATE DATA FOR STUDY OF FACTORS INFLUENCING CARBOHYDRATE UTILIZATION BY BOLLS

TREATMENT	FRESH WEIGHT			BOLLS		CARBOHYDRATES							
						LEAVES				ROOT BARK			
	LEAVES PLUS STEMS	TAP ROOTS	BOLLS	PER PLANT	PER 100 GRAMS STEMS PLUS LEAVES	REDUCING SUGARS	SUCROSE	STARCH	SUM	REDUCING SUGARS	SUCROSE	STARCH	SUM
	gm.	*gm.*	*gm.*	*no.*	*no.*	%	%	%	%	%	%	%	%
LOW LIGHT													
Fruited													
4 m.e. NO_3	319	22.2	153	11.4	3.8	0.03	0.17	0.23	0.43	0.20	0.58	0.38	1.16
16 m.e. NO_3	328	21.6	150	11.2	3.4	0.04	0.18	0.24	0.46	0.21	0.63	0.37	1.21
Debudded													
4 m.e. NO_3	417	30.1	.....	.....	.....	0.05	0.18	0.24	0.47	0.31	1.32	0.52	2.15
16 m.e. NO_3	443	29.2	.....	.....	.....	0.09	0.18	0.26	0.53	0.41	1.46	0.50	2.37
HIGH LIGHT													
Fruited													
4 m.e. NO_3	382	28.1	458	22.9	6.4	0.13	0.28	1.34	1.75	0.27	0.44	1.09	1.80
16 m.e. NO_3	367	29.1	468	22.9	6.4	0.13	0.33	1.59	2.05	0.48	1.06	1.37	2.91
Debudded													
4 m.e. NO_3	555	46.6	.....	.....	.....	0.14	0.34	1.54	2.04	0.71	1.89	1.81	4.41
16 m.e. NO_3	556	53.8	.....	.....	.....	0.15	0.43	2.04	2.62	0.68	2.28	3.90	6.86

35 per cent. of their bolls. Likewise, there was only a suggestion of a difference in the sugar or starch concentrations in the leaves between the two nitrate levels. There were, however, relatively large differences between these two nitrate levels in the sugar and starch concentrations in the root bark of both the fruited and debudded plants; i.e., more carbohydrate was passed on to the root bark by the 16-m.e. plants than by the 4-m.e. plants, indicating that the effects of light intensity on carbohydrate accumulation are not independent of substrate characteristics. Under high light, as under low light, the carbohydrate supply can be construed as having limited growth and boll production for with the floral buds removed, the plants accumulated more carbohydrate, made more vegetative growth, and developed more floral buds than did the fruited plants.

These high-light plants set twice as many bolls per plant and almost twice as many bolls per 100 grams of fresh stems and leaves as did the low-light plants and they had an average of 2.6 times as high carbohydrate concentrations in their leaves and root bark as did the low-light plants. A critical question is introduced by this observation. If carbohydrate supply limited the fruitfulness of the high-light plants, why were the carbohydrates in the high-light plants not reduced to the levels found in the low-light plants before the carbohydrate supply became the limiting factor? Evidence that the carbohydrates found in the high-light plants were not unusual is provided by the similarity with the concentrations found in plants of comparable development growing in the field.

Effect of light on fruiting dominance

Before singling light out as the dominant influence that altered fruitfulness and the level of carbohydrate accumulation between the winter and summer experiments some further attention to length of day and temperature is desirable.

As noted in the introduction, Konstantinov (**23**), though finding length of day to alter the fruiting activities of some but not all of the perennial arborescent cottons, particularly those from equatorial regions, and to a slight extent varieties of Egyptian and of medium and late uplands, concluded that the early upland cottons, as well as wild forms from Mexico and elsewhere, were without length of day reactions. When length of day reactions were found, the basic change consisted in a lowering of the position of the first fruiting branches. Lengths of day ranging from 6 to 14 hours were arranged by covering the plants with plywood boxes.

Although the mean temperatures of the summer and winter experiments were similar and it has seemed doubtful that the small and unavoidable differences could have contributed to the greater fruitfulness of the high-light plants, it is known that temperature has a notable effect on the fruiting of the cotton plant. An experiment was conducted in 1922 (**12**) near San Diego, California, where the midday summer temperatures are rarely in excess of 80° or 85° F. and the cotton plant sets few bolls and develops a pre-

dominance of vegetative branches. Using a 13-hour day, Durango cotton plants subjected to nightly temperature of 90° F. fruited well and developed no vegetative branches; whereas plants subjected to an uncontrolled nightly temperature averaging about 65° F. (also a 13-hour day), set no bolls and developed many vegetative branches.

There are fairly direct indications from other sources that light intensity, independently of air temperature or length of day, has notable effects on the relative fruitfulness of cotton. In 1935, KNIGHT (**22**) reported on the effects of shade on the development of an American upland cotton grown in field plots under full exposure and under the shade provided by a single layer of white cloth. The shade increased the height of the plants from 55 to 60 cm. but reduced the number of bolls per plant from 10 to about 5, the yield per plot from 20.8 to 7.2 pounds, and the number of fruiting branch nodes per plant from 63 to 44. KNIGHT undertook these experiments in the Sudan hoping that he might thereby account for the non-fruitful growth of cotton in an area to the south that was characterized by much cloudy weather, but he makes no statement of his conclusion.

If light intensity is, as it seems, one of the critical factors determining the dominance of fruiting activities in cotton over vegetative growth and, in association therewith, the levels at which the carbohydrate concentrations in the plant are maintained, the subject possesses a substantial physiological significance. The data at hand are not sufficiently extensive to justify much theoretical discussion but a number of questions are nevertheless involved. Prominent among these is whether high light in favoring boll growth at the expense of vegetative expansion is responsible for the formation, or inhibition, of some hormone that differentially determines the kind of growth activity that predominates, or whether the light affects directly, according to degree of exposure, the enzymes that are responsible for carbohydrate utilization. The fact that hormone activities may be local, like enzymatic activities, adds to the difficulties involved in a decision. It is nevertheless desirable to consider the fact that the meristematic tissues of the vegetative structures are more extensively exposed to light than are the floral buds and the contents of the boll. The involucral bracts that enclose the floral bud effectively protect it from direct light and following anthesis the massive structure of the boll likewise derives from the bracts a substantial measure of protection. This question is obviously complex but measurements made by KEARNEY (**20**) would seem to have some bearing on the subject. He found that developing Pima cotton bolls, which are protected by especially prominent involucral bracts, were caused to shed in larger numbers and the bolls that were retained to be smaller when mature, when the involucral bracts were cut away at or before anthesis than when they were left intact.

NUTRITIONAL FACTORS AND HORMONES IN RELATION TO THE DEVELOPMENT OF VEGETATIVE BRANCHES AND THE SHEDDING OF FLORAL BUDS AND BOLLS

The vegetative branches of the cotton plant, which closely resemble in their morphology (**5, 6**) the main stalk, develop under favorable conditions

from the axils of the leaves of the main stalk, of other vegetative branches, and of the fruiting branches. Fruiting branches may also arise from any of these positions even though, like the vegetative branches, their development may be limited to a single internode.

COOK and MEADE (**6**) have pointed out: "Weather that is dry enough to retard the growth of plants also discourages the formation of limbs and vegetative branches while humid conditions favor the production of both." In the present investigation vegetative branches were produced to an appreciable extent only by the debudded plants supplied with 4 m.e./l., or more, of nitrate under high light. These plants had higher concentrations of carbohydrate and nitrogen than the plants under other treatments and, on the basis of this evidence, it would be reasonable to assign high carbohydrate and nitrogen supply, together with favorable moisture conditions, as causes for their development and growth.

Following methods used by HITCHCOCK and ZIMMERMAN (**18**) in their experiments with tomato plants, the writers have secured evidence that hormone as well as nutritional factors may be involved in the development of vegetative branches. When black cloth bags were placed over the terminal 4 or 5 inches of cotton plants growing in the field during the summer no measurable increase in branch formation resulted, but when the same bags were used on potted plants growing in the full light within a greenhouse during the winter the responses were clear cut and unmistakable. The extensive development of vegetative branches that followed the application of the bags in the greenhouse experiment is illustrated in figure 8. The cloth used for the bags was not entirely opaque and when stretched over the cell of a photometer and exposed directly toward the sun it transmitted 600 f.c. of light. Too much light for this response may have passed through the cloth under the summer conditions, but other explanations for the lack of response during the summer are not excluded. As mentioned in the previous section, the development of fruiting branches has been found in other investigations (**12**) to be repressed and the formation of vegetative branches to be greatly stimulated when day temperatures were mostly below 80° F., but temperatures of 90° F. provided during the night reversed this behavior.

The present information does not permit of any well defined application of observation, or theory, to relations between hormone activity and the shedding of cotton bolls, but some of the marked differences between cottons in the extent to which shedding occurs, would seem to preclude the possibility that in all cases this shedding can be attributed solely to nutritional relations. As previously noted the 4- and 16-m.e. plants under high light produced like numbers of bolls, and shed like numbers and yet the concentration of sugars in the root bark of the latter (also the 64-m.e. plants) was substantially higher than in the 4-m.e. plants. It has been noted (**6**) that certain Asiatic cottons have not been fruitful when introduced into the United States and such cottons have been found to be more fruitful when exposed to short days (**23**) than to long days. Heritable differences (**21**) in

shedding rates have been recorded and many hybrid plants have been observed to be unproductive. Nutritional relationships are indicated, on the other hand, by the well known fact that large bolled upland cottons shed more but produce more seed cotton than does the small bolled Pima Egyptian cotton which retains a greater proportion of its bolls. EWING (**16**) has likewise recorded differences in the rates of shedding among upland cottons with different boll sizes.

FIG. 8. Stimulation of vegetative branch formation by capping terminal growth with black cloth bag. At left, capped plant with 4 branches (av. of 6 plants, 2.7); at right, control plant with 1 short branch (av. of 6 plants, 0.7). All leaves except those on the vegetative branches have been removed.

In direct accord with the carbohydrate concentrations found in the plant tissues there was an extensive shedding of very small floral buds by the fruited plants at all nitrate levels under low light, whereas the shedding of floral buds was negligible from the debudded plants (buds removed when much larger) under low light, and from both the fruited and debudded plants under high light. The fact that the debudded plants under low light

did not shed their buds goes far to eliminate the possibility that such shedding was a hormone or length-of-day response characteristic of a specific set of external conditions. Specific effects of high or low nitrogen supplies are likewise eliminated, since the low-light fruited plants shed their squares extensively at all nitrate levels. On the basis of the foregoing correlation between carbohydrate levels and square shedding a reasonable nutritional explanation is afforded, but additional causes under other conditions are not eliminated and an extensive shedding of floral buds sometimes occurs in the field (**1, 25**). Following the initiation of abscission floral buds usually remain in place until they have turned brown and been disengaged by the enlargement of the pedicel sockets.

In endeavoring to account for the intermediary mechanism between nutritional factors, or hormone activities, and the shedding of floral buds and bolls of cotton, it is worth while to remember that with the development of fruiting branches a non-fruitful condition can exist only as the result of abscission. A final question must exist accordingly as to whether the stimulus responsible for the behavior of the cells in the abscission layers acts by preventing this separation or stimulates these cells into separation.

Carbohydrate-nitrogen ratios[3]

It has seemed to the writers that the concept of carbohydrate/nitrogen ratios, wherein the actual magnitudes of the contributing values are lost sight of, is a matter quite apart from the significance of carbohydrate and nitrogen concentrations considered as such, either alone or in conjunction with one another. The latter relations have aided an interpretation of the foregoing experiments but it has not been possible to derive much benefit from the ratios of these two variables. As pointed out by others in the past, there has been some confusion in the interpretation of carbohydrate and nitrogen data due to difficulties in differentiating between causes and effects.

Whether attention is directed to the carbohydrate/nitrogen ratios that may be calculated separately for leaves and root bark or to the averages of these concentrations from which the ratios in table V are computed, the results show that these ratios were influenced to nearly as great an extent by fruiting itself as by any of the treatments that altered fruitfulness. Observations repeated in many experiments with cotton prompt the belief that all of the debudded plants, irrespective of their carbohydrate-nitrogen

[3] When this manuscript was returned by the editor for the authors' review it carried the following signed comment along the margin of the first paragraph under the above heading: "I have never been able to see, nor do I now see, value in a C/N *ratio* as a possible means of expressing a causative factor concerned with flowering of plants, despite the fact that I am *supposed* to be one of the proponents of the idea. Hence I agree here. Even to decide the meaning of the presence of C and N is very difficult and to establish such a simple *ratio* seems impossible to me. The most one may hope for is to determine the possible interrelations of the carbohydrate and nitrogenous constituents of plants and the possible meaning of such relations in reference to the vegetative, reproductive, or fruiting status."—E. J. Kraus. This statement is repeated here with Dr. Kraus' approval hoping thereby to clear up a misconception and otherwise serve a useful purpose.

ratios, would have promptly set bolls if the debudding had been stopped at any time, and, likewise, that the removal of previously set bolls from any of the fruited plants would have been followed by the setting of new bolls to replace them.

It is true that the carbohydrate/nitrogen ratios of the fruited plants were substantially lower under low light than under high light and that the latter plants were most productive. On the other hand, through the four nitrate concentrations supplied to the fruited plants of the low-light experiment the trend of the ratios was consistently downward, whereas in the high-light experiment this trend was upward, and yet in both experiments the relative fruitfulness of the plants supplied either with low or high nitrate was somewhat greater than that of the plants supplied with intermediate concentrations. Some of the dominance of fruiting activities over vegetative growth that was found under high light was lost under low light but this reduction in fruiting dominance was not materially altered by nitrogen supply.

Had the investigation been extended to include measurements of potassium and phosphorus accumulation, a further insight might have been gained, as pointed to by others, on the relation of nitrate supply to the accumulation of these elements and the effects of phosphorus, in particular, on carbohydrate transformations.

Nitrogen supply and fruitfulness under field conditions

The general accord between vegetative growth and fruitfulness of cotton under varied nitrate supply as found by many investigators, as well as in the present experiments, raises a point of significance in regard to the cause of the non-fruitful cotton plants that have sometimes been observed on rich moist soils. These non-productive, rank-growing plants have been referred to variously as "he cotton," "bull cotton," and "cotton gone to weeds." Although the growth of such plants is sometimes regarded as being due to an overabundance of nitrogen, direct evidence seems to be lacking that nitrogen supply is in itself the cause. If excessive nitrogen can be an immediate cause of non-fruitfulness in cotton it would seem that someone would have observed it in connection with the hundreds of nitrogen fertilization experiments that have been conducted in the cotton producing regions. Insofar as the writers could determine from their search of the literature, no such observation has ever been recorded and there was no evidence of such a relationship in the present experiments. An experimental demonstration that a non-fruitful growth of cotton can be brought about, under conditions otherwise suitable for boll setting, by any arrangement of nitrogen supply or of carbohydrate/nitrogen ratios would attract great interest. In the absence of a conclusive demonstration of this character it is more reasonable to look for the cause of marked unfruitfulness in other directions. A rank growth can occur only with an abundant nitrogen supply but that does not mean that extra nitrogen is in itself a cause of nonfruitfulness.

It is extensively believed that cotton stimulated in heavy vegetative

growth by abundant supplies of nitrogen suffers more severely from drought than when growth has been retarded. On the other hand, determinant strains of cotton have been observed by DR. J. W. NEELY at Stoneville, Mississippi, to be less able to withstand mid-summer drought than ranker growing less-determinant strains planted nearby. It seems probable that the latter cottons, by reason of the greater root development that is associated with fewer early-season bolls, more fully occupied the deeper zones of the alluvial soil and thereby obtained moisture that was not available to the determinant cottons.

A rank but unproductive growth of cotton can in some instances be attributed to the activities of the flea hopper, *Psallus seriatus* Reut. This bud-destroying insect is of special interest in the present connection for the reason that it destroys the floral buds while they are very small (**31**) leaving no evidence of the insect activity except for the vegetative condition of the plants. It has been pointed out (**33**) that succulent cotton growing on rich moist soil is especially attractive to flea hoppers and the destruction of the floral buds would contribute directly to an increased vegetative expansion of the plant. The flea-hopper suggestion does not exclude other explanations. In their study of the shedding of floral buds associated with wet soils, ALBERT and ARMSTRONG (**1**) called attention to a similarity of the resulting plants to flea-hopper injured plants but emphasized that this insect had not been found to be present.

The plants of the present experiments were supplied with what were regarded as ample concentrations of potassium, phosphorus, boron, etc. There is a possibility that a non-fruitful condition might result from abundant nitrogen in the presence of limiting concentrations of other of the essential elements. Something of this character has been observed in the instance of boron but otherwise no very concrete evidence that such is the case has been found in the literature. In the instance of boron deficiency, however, the shedding of floral buds was associated with a marked shortening of the length of the fruiting branches.

Summary

The foregoing work has shown that the level of nitrate supply to the cotton plant has, in itself, no outstanding effect upon fruitfulness relative to plant size. An increase in nitrate from a low level resulted in an increase in vegetative growth and in an increased number of bolls. A toxically high concentration of nitrate reduced growth and reduced the number of bolls. These relations were found to exist both in an experiment conducted in the greenhouse during the short days of winter under muslin shades (midday light intensity about 1,000 foot candles) and during long summer days under free exposure outdoors (midday light intensity about 10,000 foot candles). The plants were grown in sand cultures supplied with nutrient solutions in sufficient excess to produce 25 to 50 per cent. of drainage.

The nitrate was supplied in nutrient solutions in concentrations of 1, 4,

16, and 64 m.e. per liter (14, 56, 224, and 396 p.p.m. of nitrogen). At both light intensities the plants on low nitrate (1 m.e.) were less than half as large as those on 4 and 16 m.e. of nitrate, and the plants on high nitrate (64 m.e.) were about 75 per cent. as large. In both experiments the relative fruitfulness values (number of bolls per 100 grams of fresh weight of stems and leaves) for the 1- and 64-m.e. plants were actually a little higher than the values for the plants supplied with 4 and 16 m.e. Under low light these values were 4.46, 3.47, 3.36, and 4.09; and under high light 6.75, 6.42, 6.43, and 7.58 for the four respective nitrate levels. Relative to their fresh weights the 1- and 64-m.e. plants differentiated more nodes and flower buds than did the 4- and 16-m.e. plants and their shedding percentages were higher.

At the respective nitrate levels, the fresh weight of stems and leaves in the low-light experiment were nearly the same as the corresponding weights under high light, but the high-light plants were far more fruitful. The mean number of bolls per plant at the 4 nitrate levels in the winter experiment were respectively 5.7, 11.4, 11.2, and 9.6 and in the summer experiment 7.2, 22.9, 22.9, and 18.4. Fruiting activities thus exercised a much greater dominance over vegetative growth under high light than under low light. The mean temperatures in the two experiments were similar. It has been shown by others that relative length of day has little, or no, effect upon the fruiting of American upland cottons.

Each of the experiments included two series of plants: (1) fruited plants and (2) plants from which all floral buds were removed as these attained a bract width of about 15 mm. The debudded plants were included to aid a differentiation between the effects of treatment and effects of fruiting upon growth and upon carbohydrate accumulation and utilization.

Debudding resulted in an increased number of main stalk and fruiting branch nodes, and resulted in a large increase in the weight of stems and leaves. This increase in weight of stems and leaves was much less in the low-light experiment than in the high-light experiment which is in accord with the lower weight of bolls in the low- than in the high-light experiment.

High nitrate was more toxic to the fruited than to the debudded plants.

On the basis of a factorial summary, carbohydrate concentrations were lowest in plants supplied with 4 m.e. of nitrate, lower in fruited than in debudded plants, lower under low light than under high light, and lower in the leaves than in the root bark. The interactions between treatments altered some of the foregoing effects.

Shedding of very small floral buds was a prominent feature in the growth of the fruited plants under low light (all nitrate levels) but bud shedding from fruited plants under high light was negligible, and it was also negligible from the debudded plants (buds removed when much larger) under both light intensities. Square shedding thus occurred only from the plants with the lowest carbohydrate concentrations.

The 64-fold increase in the nitrogen concentration in the substrate tended

to double the nitrogen content of the leaves and quadruple the concentration in the root bark.

Higher nitrogen concentrations were found in the debudded than in the fruited plants and also under high light than under low light. This is in accord with the extent of carbohydrate accumulation in the root bark and it is in agreement with results previously reported that supported a metabolic explanation of the seasonal trend of nitrogen levels in the cotton plant.

The data on fruitfulness and on carbohydrate and nitrogen accumulation afford little or no basis for attaching any special significance to carbohydrate/nitrogen ratios in the cotton plant. Fruiting itself had effects on this ratio as great as the nitrate supply. The actual magnitudes of the carbohydrate and nitrogen concentrations, however, were both informative.

In both experiments it appeared that limited carbohydrate supply was responsible for much of the boll shedding. However, the high-light plants contained sugar and starch concentrations (average of leaves and root bark of fruited and debudded plants at all nitrate levels) 2.7 times as high as the low-light plants. The fact that carbohydrate concentrations were not reduced in the high-light plants to the levels found in the low-light plants before shedding occurred is interpreted as indicating that high light sets in motion some factor that depresses carbohydrate utilization. Fruiting activity had a greater dominance over vegetative growth under high light than under low light.

Bureau of Plant Industry, Soils and Agricultural Engineering
Texas Agricultural Experiment Station
College Station, Texas

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STARCH AND SUGARS OF *VITIS VINIFERA*[1]

A. J. Winkler and W. O. Williams

(WITH SIX FIGURES)

This investigation on the carbohydrate values within *Vitis vinifera* is included in a general study of nutrition. Presumably, a better understanding of the physiology of the grapevine would result in improved viticultural practices. It would serve as a suitable basis for evaluating the analytical results obtained by studying the carbohydrate nutrition of the vine and also for interpreting data on pathologically affected vines. Certain points were pertinent. Investigators have often suggested that carbohydrates are translocated during the dormant period. If this translocation does take place, its bearing on the time of pruning would be important. Neither Richey and Bowers (**19**) nor Schrader (**22**) found such translocation in the Concord grape. It would, indeed, appear unlikely; the callus closure of the sieve plates during the dormant season seems to render the accepted channel of elaborated food transport unavailable. Another important point was the significance of hemicellulose as a reserve food. Data on this have already been published (**35**). There were no indications that hemicellulose was available as a reserve in the grape even under the severe deficiency conditions resulting from repeated defoliation.

A third point was brought forth by findings such as those of Davis (**5**) with the sugar prune. According to Davis, there is a comparatively large spring increase of starch in the bark and wood of the spurs—an increase not explained by the decrease in sugar content. Richey and Bowers (**19**) and Schrader (**22**) also found that the total carbohydrate content of Concord grape stems became markedly greater during the dormant period. Such findings might hypothetically be explained by one or several assumptions: (1) by transformation to or from other materials, such as fat, hemicellulose, or unknown substances; (2) by transport of sugars from another part of the tree; or (3) by sampling and analytical error. Davis in an unpublished analysis of the same material could not account for this increase by the disappearance of hemicellulose. The data of Richey and Bowers (**19**) and of Schrader (**22**)presumably include the hemicellulosic fraction in the total carbohydrates. A potential source of such materials might be a fatty reserve; and there remains the possibility of such unevaluated substances as Swarbrick (**28**) and Sinnott (**24**) have observed in microchemical studies, or some fraction of the comparatively great amount of nonsugar alcohol-soluble substance observed by Niklewski (**16**). Schrader (**22**) has found much of this soluble nonsugar material in Concord grape shoots. According to Smyth (**27**), an extremely large quantity of it occurs in young apple wood. The improbability of the translocation hypothesis has already

[1] This is the second of a series of papers on the carbohydrate metabolism of *Vitis vinifera*.

been pointed out. The sampling and analytical errors have often been large, judging from the irregular variations found in many biological data. One must be especially careful, it seems, in interpreting variations, to make sure that they do not result from transient environmental factors or individual variations. Care is especially important when one must sample small populations, as in many horticultural investigations.

There has been a lack of analytical studies on the annual cycle of fatty content; the observations have been mostly microscopic. Judging from the data of NIKLEWSKI (**16**) and DU SABLON (**20**), however, the fluctuations in fat do not account for the often-observed variance in the summation of winter carbohydrate content. HOOKER (**9**) has found considerable fat in apple shoots. NIKLEWSKI (**16**) believed fat formation to be independent of the starch-sugar change. Conceivably, however, other substances may act as intermediaries, so that an indirect relation may be obscured by variations in the amount of the transient intermediary substance. Probably, in view of the normally observed increase of fat with the starch decrease, as found by TUTTLE (**30**) in microchemical tests, the fat fluctuation at least partially explains the observed discrepancies. Respiration losses may account for much of the starch that does not reappear as sugar. Although petroleum ether extracts of representative grape tissues indicate that the fat-soluble material of the bark samples is somewhat higher in the active growing season than in fall and winter, the absolute magnitude of the change is relatively insignificant. The change in the wood samples is even smaller. The grapevine appears to furnish the opportunity for ascertaining whether or not there is actually some heretofore unrecognized storage substance without the interference of fat storage.

Collection and preparation of material

The procedure for this investigation consisted in removing composite samples of ten vines (only eight in the last three samplings) at appropriate intervals throughout the year. The vines employed—*Vitis vinifera* var. Carignane—were just completing their fourth annual growth ring in the trunk at the beginning of the sampling period. During the year, eighteen collections were made. The first, on Nov. 6, came just before leaf fall. The vines taken were distributed by removing every twentieth vine throughout the plot, omitting end vines and those of abnormally weak or strong development. The shoots (the term here indicates the current season's growth) were cut between 7 and 8 A.M. and taken directly to the laboratory. The leaves were promptly stripped off, and comparatively large samples of young and of mature leaves were taken. Then 4- to 6-inch sections were removed from the base, middle, and tip of each shoot. The base and middle sections were divided into wood and bark. All samples were quickly cut into small pieces, weighed, and rapidly dried in a small fruit dehydrater with a very strong air blast at 60° to 63° C. The vines were cane pruned so that mature shoots (canes) could be sampled in the same manner, except that there were

no tip samples. Two 3-inch sections were sawed or cut from each trunk, the bark was removed, and the wood was separated according to annual rings: one-year-old wood and the subsequent growth during the sampling period, two-year-old wood, and wood three years old and older (two years' growth, including the pith). As soon as the trunk was removed, the roots in the vicinity of the vine were dug up. After washing and drying of the external moisture, three types of root samples were taken: small rootlets (3/32-inch and less), one-year-old roots (3/16- to 1/4-inch), and two-year+ roots (1/2- to 3/4-inch). The one- and two-year root sections were divided into wood and bark samples. The dried samples were ground in a Wiley mill.

Analytical procedure

To obtain the sugars, the samples were continuously extracted with 95 per cent. ethyl alcohol for 6 hours by letting the condensed alcohol fall directly from the condenser onto the sample contained in alundum thimbles, the liquid level being kept above the material to help prevent channeling. The alcohol extract was evaporated to a small volume. The residue, taken up in warm water, was subsequently treated with an excess of neutral lead acetate, and the precipitated material was centrifuged off. The excess lead was removed with oxalate. Invertase was used for the sucrose inversion. The alcohol-extracted residue served as the sample for determining starch. The sample, plus 50 cc. water, was placed in an enclosed steam bath for one hour. Subsequently, 10 cc. of a phosphate buffer of pH 5 (**4**) and 10 cc. of the Taka diastase solution (2.5 per cent.) were added. Taka diastase utilized was purified, to remove most of the blank, by precipitation from a saturated water solution through the addition of 2.5 times its volume of 95 per cent. ethyl alcohol. The precipitate was centrifuged off and redissolved. The sample was then transferred to a porcelain jar, several flint pebbles were added, and the whole was rotated on its axis for 16 hours at 250 r.p.m. This procedure reduced the material to a very fine state of division, facilitating complete hydrolysis. If starch was revealed by a microscopical examination, the samples were re-treated. The extract was clarified, using lead acetate, and brought to a definite volume; and sufficient hydrochloric acid was added to make 2.5 per cent. by volume. The samples were then hydrolyzed in an enclosed steam bath for 2.5 hours. To determine the sugar, the SHAFFER-HARTMAN (**23**) method was used, except with the alcoholic extract from samples containing bark. In these latter samples the copper precipitated had to be filtered off and weighed; otherwise, results would have been erroneously high because of iodine reduction. This limitation of the SHAFFER-HARTMAN method for certain plant extracts has been pointed out by HARVEY (**8**), LINCOLN (**13**), and SMYTH (**27**).

Results

The data for the mature wood tissues are presented on a residual (starch- and sugar-free), dry-weight basis. This basis should indicate changes in the

amount of a variable constituent within the cells of a mature tissue more accurately than either a dry- or green-weight basis. Before calculation to a starch- and sugar-free basis, the hemicellulose (**35**) percentage showed a seasonal fluctuation inverse to that of the starch + sugar fluctuation. This fluctuation was introduced, apparently, by changes in dry weight of the cells, brought about by storage or by utilization of reserve carbohydrates. The use of a cellulose basis is theoretically perhaps more desirable. It is subject, however, to errors, resulting from the limitations of the present methods for determining cellulose; besides, it involves an additional determination.

Certain authors have stressed the particular significance of a green-weight basis believing, apparently, that such procedure gives a measure of the actual concentration within the cell. In approaching the ideal as closely as possible, however, it seems undesirable to induce further error by including the mass of extraneous solid materials that certainly do not function as solvent agents. Instead, the data might well be expressed on a concentration basis; that is, as a percentage of the water present. Both these methods introduce sources of error which should be kept in mind: (1) possibly greater variation in the moisture determination, especially where separations of wood and bark are undertaken, with consequent difficulty of preventing water loss; (2) transient variation of water caused by changing environmental conditions; (3) a localization of concentration in the tissue, no doubt also occurring in the various constituent regions of the individual cell; (4) the presence of water containing little, or none, of the substance under consideration, such as the bound water of hydrophyllic colloids. A relatively important fraction of such water may well be contained in the thick cell walls of the xylem elements, especially in the grapevine.

To present the data of reserve constituents on a starch- and sugar-free, dry-weight basis would seem more satisfactory *for mature tissues, at least for changes occurring within a given tissue.* In such mature tissue the water-, starch-, and sugar-free fraction is doubtless more constant than the water content; hence the results expressed in terms of that fraction offer a more nearly exact basis for comparison. If the changes occurring within the living cell are to be obtained, some constant quantity must serve as an index of intercomparison; it has not been feasible to analyze a definite number of cells, thus obtaining values on a per cell basis.

In the succulent, rapidly growing stem tissue, on the other hand, growth and differentiation lead to a constant alteration in the amount of residual wall substances (chiefly cellulose, lignin, and hemicellulose) contained in a given cell; thus the number of living cells represented in a gram of residual (starch- and sugar-free) dry-weight material will be continuously altered. Obviously, one cannot, on a starch- and sugar-free dry-weight basis, accurately measure the change in a questioned constituent within a single cell of such tissue. The use of a concentration basis that depends upon the total water present should be more satisfactory in succulent tissues, since

the water content must vary, at least roughly, in proportion to the number of living cells in such material. Further, the storage of insoluble reserve carbohydrates ordinarily does not become important in this period. For these reasons, some of the data are calculated in this manner, although for comparison they are also presented on a carbohydrate-free basis.

Reports by SMITH (**25**), SAMPSON and SAMISH (**21**), and DENNY (**6**) emphasize the desirability of using a unit organ basis such as a fruit, a leaf, or some other section. A basis of this kind, though doubtless better than a dry-weight basis in immature and meristematic tissues, cannot be relied upon to show changes that occur within a living cell of the tissue. The laying down of a given constituent in new cells, for instance, may obscure a decrease of this material in the older cells or may lead to the incorrect assumption that this constituent increases in the older cells.

CRITICAL DATES IN THE ANNUAL CYCLE

Although the yellowing leaves were still on the vines on November 6, the date of the first sampling, frost severe enough to remove them occurred within the same week. The buds began to break during the third week in March. Exudation of the vine at Davis, California, normally occurs for 2 weeks before bud break, indicating the start of root activity about March 1. On May 24 the flower clusters were in full bloom. Rapid shoot growth, characteristic of the properly pruned grapevine, ceases by early July. A comparison of the relation among berry growth, sugar increase in the berries, trunk circumference, and shoot length increase for this variety has been published (**36**). Since a heavy frost occurred early in October, the leaves were killed almost a month before the date of the killing frost the previous year; and no leaves were available for the last sample taken November 6, a year after the first sampling. The abnormally early loss of the leaves, together with a larger crop, may explain the lower levels of starch and sugars in the various vine parts during the latter part of this season as compared with the figures for the previous year.

Discussion

The carbohydrate data are always expressed in terms of glucose, because this method allows direct comparison on an equivalent basis.

CHANGES IN CARBOHYDRATES DURING DORMANCY

Both the reducing and sucrose sugars (figs. 1, 2, 3, 4) in the mature above ground sections of the vine increased greatly from November 6 to December 6 and climbed slowly to a maximum value January 8. Then followed a slow decrease to February 10 and a rapid decrease to March 9. During this same period, approximately opposite changes occurred in the starch content. This circumstance may be understood most readily by reference to the starch + sugar values, which show that throughout this period the summation of these carbohydrates remained essentially constant, even though indi-

vidually each had undergone large fluctuations. There have been many investigations, mostly microchemical, of the winter disappearance and reappearance of starch. The papers of TRAUB (**29**) and SWARBRICK (**28**), as well as the earlier investigation of PRESTON and PHILLIPS (**18**), give reviews of the literature. The data confirm the general finding of SINNOTT (**24**) that the starch does not completely disappear during the dormant season in the plants he classifies as "starch" or "nonfat" trees. Perhaps with the very low starch storage that seems to occur in many "fat" trees, practically all the starch would disappear, for the sugar quantity produced may "control" the hydrolysis.

Since there was no increase or excessive decrease of carbohydrates in any of the "mature" stem sections, the theory of food transport through the dormant tissues is untenable in the present case. The results, together with the commonly observed winter callus deposit on the sieve plates of deciduous plants, indicate the improbability that any general transport of elaborated food occurs during the dormant season. TRAUB (**29**) has noted the lack of available information on the transport of material in dormant apple twigs. In the vine, the winter disapparance of starch can be accounted for almost completely by the appearance of an equivalent amount of sugars. This intraconversion, with the high degree of constancy shown in the summation of the material involved, fails to indicate the presence of storage materials in unknown forms, of fats, or of a labile hemicellulose reserve in the vines.

Contrary to the relation suggested by SINNOTT (**24**), our results on the water level of the vine parts (unpublished data) fail to show a related variation in the water content during the carbohydrate metamorphosis in the dormant period.

There was no indication that starch was converted to sugar in the root sections during the early winter. The content of reducing and sucrose sugars remained fairly constant, although the starch content underwent comparatively large fluctuations (figs. 2, 3). Probably, the variations in starch arise from the difficulty of obtaining representative samples of the root sections, combined with utilization by growth and by a generally more rapid rate of respiration at the higher temperatures in the soil. PRESTON and PHILLIPS (**18**) reported a winter decrease of starch in the roots of only one out of nine tree species studied, though the stems of all species showed the conversions of starch to sugars in winter. SINNOTT (**24**) and WIGHT (**32**) also found little reduction in the starch content of the roots during the dormant season. The data of JONES and BRADLEE (**11**) indicate a conversion of starch in the roots of the sugar maple.

The maximum values of the sugars and the minimum values of the starch contents for the *dormant period* are given for the bark and wood (table I). These values occur simultaneously on January 8. As a whole, the data do not indicate any significant relation between the extent of the conversion of starch to sugar and the total content of starch, reducing sugar, or sucrose. When, however, the comparisons are limited to more nearly comparable

TABLE I

MIDWINTER (JAN. 8) STARCH AND SUGAR DATA FOR STEM WOOD AND BARK SECTIONS

	CONSTITUENTS AS PERCENTAGE ON CARBOHYDRATE-FREE, DRY-WEIGHT BASIS							
	IN THE WOOD					IN THE BARK		
	MIDDLE CANES	BASAL CANES	1-YEAR TRUNK	2-YEAR TRUNK	3-YEAR+ TRUNK	MIDDLE CANES	BASAL CANES	TRUNK
	%	%	%	%	%	%	%	%
Starch	11.3	12.2	14.9	15.2	14.3	2.8	3.6	7.9
Sucrose	4.6	5.1	4.5	4.6	3.6	8.8	7.5	8.3
Reducing sugar	5.6	6.3	4.2	3.8	3.9	6.1	7.2	7.6

sections—that is, when comparisons are made between the two cane sections and the trunk sections, or between the bark sections—there seems to be an approximate constancy in the percentage of either sugar produced. The generally higher values in the bark probably result chiefly from a lower residual dry weight per living cell. Only rough, qualitative comparisons should, however, be made between different sections; there is thus far no adequate basis for quantitative comparisons.

Judging from the rough constancy in the quantity of sugar produced, the rate of a counteractivity of synthesis has been favored by increasing amounts of the substrate. Unpublished data indicate that the conversion of starch to sugar proceeds slowly, if at all, after a certain point is reached, even though the material is held at low temperatures for an extended period. The relatively equivalent quantities of reducing and of sucrose sugar (table I) agree with TRAUB's figures (**29**) on entire apple twigs during the winter. But SMYTH's (**27**) data on apple twigs and those of JONES and BRADLEE (**11**) indicate a rough ratio, the content of reducing sugar being about 1.5 times that of sucrose during the dormant season. The approximately equivalent quantities produced by the hydrolysis may result from a coincidental point of equilibrium between the two sugars. The common failure to find a definite relation between the amounts of sucrose and of reducing sugars resulting from winter hydrolysis of starch apparently points to such a coincidence in the present case.

TABLE II

AVERAGE VARIATIONS IN THE SUMMATION OF CARBOHYDRATES DURING THE DORMANT PERIOD

	CANE TIP	MIDDLE CANES	BASAL CANES	OUTER TRUNK	2-YEAR TRUNK	3-YEAR TRUNK	2-YEAR ROOT	1-YEAR ROOT	ROOTLETS
Changes per month in wood	−0.5	−0.2	−0.4	−0.9	−0.5	−0.2	−0.9	−3.9	−1.8
Changes per month in bark	.....	+1.3	+0.2	−0.3	.....	.....	−0.9	−3.8	.....

Dormant period utilization of carbohydrates

During the dormant season there is a small, irregular decrease in the total summation of carbohydrates. Doubtless this decrease results from the respiration of the plant. Winkler and Shemsettin (**34**) found no perceptible bud development during the dormant period. Table II shows the algebraic averages of the individual differences per month during the 4-month dormant period (Nov. 6 to March 9) for the various wood sections and during the 3-month dormant period (Nov. 6 to Feb. 10) for the bark sections. Only a 3-month period was used for the bark, since the decreases in the carbohydrates of the bark of the middle cane section indicated growth activities between February 10 and March 9.

The average decrease in the carbohydrates of the wood of the aboveground stems as a result of winter respiration was about 0.5 per cent. per month. All the monthly differences show irregularities. This is true, especially, of the bark samples; hence the figures are only a tentative measure of the respiration losses. In this connection, the findings of Wager (**31**) and of Beaumont and Williman (**1**) on respiration during the dormant period would indicate an excessive rate of carbohydrate loss from their material. Approximate calculation from their data, with conversion of carbon dioxide collected to an equivalent amount of glucose, indicates that all the stored labile carbohydrates would be utilized in respiration during the dormant season. Such attempts to measure respiration by means of the carbon dioxide evolution appear to be misleading.

There is a larger carbohydrate decrease in the young roots. Aside from the probably greater sampling error in these sections, this decrease may result from a higher respirational rate (brought on by a higher average temperature of the soil as compared to the air) and from root growth during the winter period. The supposition of root growth is favored by the relatively slow decrease in the large roots, presumably under comparable edaphic conditions, especially of temperature. Spring utilization in the sugars and starch are obscured by the persistence of the conversion of sugar to starch. For this reason, the summation of the starch + sugar will be used for the comparisons during this period.

In the "matured" bark sections of the stem, the first large decrease of carbohydrates seems to have occurred in the bark of the middle of the canes on March 9 (from 17.6 per cent. on Feb. 10 to 12.0 per cent. on March 9), followed on March 17 by decreases in both basal cane and trunk bark (fig. 3A). The two-year root bark did not show a sharp decrease until 2 weeks later, March 31 (fig. 3C). The bark of the one-year roots seems to show a more or less continuous decrease during both winter and spring (fig. 3D). The rapid decrease did not occur in the rootlets until after April 11 (fig. 3D).

The early utilization of the starch + sugar of the "mature" wood was irregular. The trunk wood sections all showed rapid decreases by March 31 (figs. 1C, 1D, 2A) whereas the basal wood of the canes did not show a

marked decrease until April 11 (fig. 1B) and the middle cane wood not until April 22 (fig. 1A). Judging from these figures, the rapid removal of

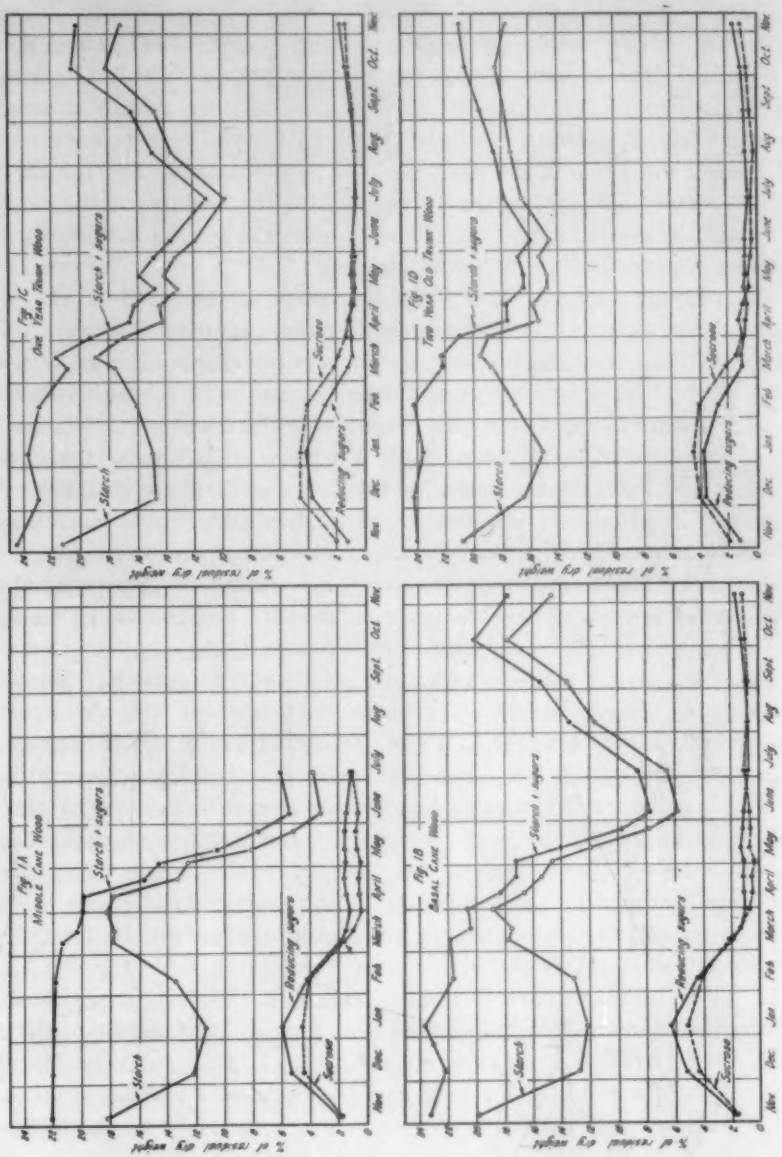

FIG. 1. Graphs showing the seasonal variation of the labile carbohydrates in mature wood sections of grapevines. (A) The middle of the canes; (B) the base of the canes; (C) the one-year trunk; and (D) the two-year-old trunk.

carbohydrate begins in the trunk and progresses upward. Such a depletion could be explained by assuming upward transport in the transpiration stream, since a progressive upward depletion, such as occurs in a counter-

current extraction process, would be the expected result. Starch disappearance between girdled trunk sections seems not to be prevented by removal of the bark and destruction of the cambium as well as the superficial layer of cells to prevent callusing (unpublished data). Such a mechanism of upward transport by the transpiration stream is opposed, however, by unpublished evidence that the inner rings of the grape stem normally function indifferently, if at all, for water conduction; yet the inner and outer sections seem to lose their carbohydrates more or less simultaneously. Furthermore, the period of marked decrease in the two-year + root wood follows behind that of the one-year trunk.

Relative storage of carbohydrates in different vine parts

The carbohydrate (starch + sugars) content of the bark increases severalfold in going downward from the canes to the one-year roots (fig. 3). In the one-year root bark only 16 per cent. of the dry weight consisted of materials other than starch and sugars. The storage of carbohydrates in the older root wood was less than in the stem wood (figs. 1, 2). Except for the November 6 sample, still less storage occurred in the one-year root wood. The high storage of reserve carbohydrates usually found in roots appears, on the basis of the present data, to be chiefly confined to the root bark. The rootlets, consisting of both wood and bark, exhibited an intermediate carbohydrate content (fig. 2D).

The greater percentage storage of carbohydrates in the root bark appears useful in providing for root growth during the absence of leaves, for root growth during the active development of shoots in the spring, and for reserves until storage again begins, since storage starts much later in the root tissues than in the stem. The minimum content of carbohydrates did not occur in the roots until sometime after storage had begun in the stem tissues (figs. 1, 2). In unfavorable years, conceivably, meager storage may occur in the roots, making useful the comparatively large accumulations stored in the more favorable years. The large difference in the amount of starch storage in these tissues at the first and last samples taken supports this hypothesis.

In comparison with the stem, the root tissue is structurally weaker and has a decreased hemicellular content (**35**), indicating a decreased proportion of cell to cell wall, which would be reflected in a higher percentage of carbohydrates even though the individual cell content were constant. Although part, at least, of the difference between the carbohydrate contents must have resulted from the basis of comparison used, other factors, such as an increased rate of enzymatic synthesis of starch, apparently may cause the laying down of the extremely high starch content of the root bark. Some such more active enzyme relation may be indicated by a lower sucrose content of the root-bark sections (fig. 3) during the fall storage period, as compared with the aboveground bark sections. The higher average temperature of the soil as compared with the air in autumn (**25**) may promote a more active accumulation of reserves.

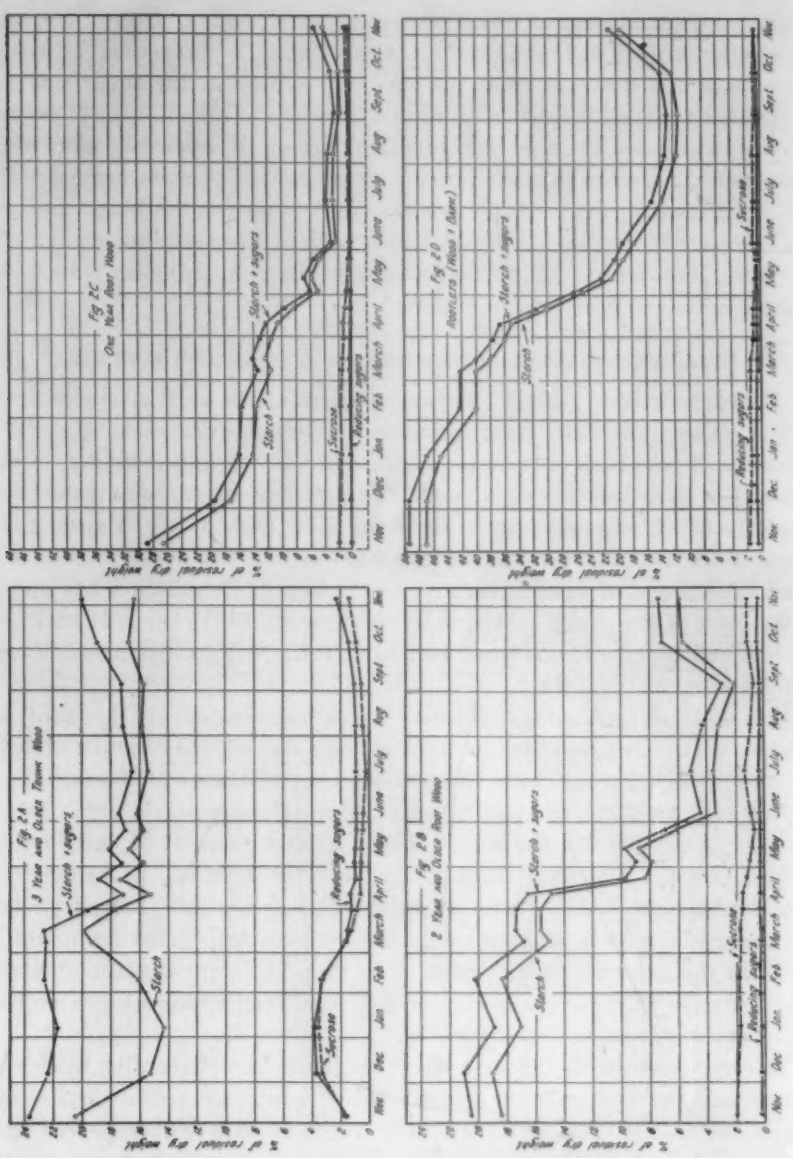

FIG. 2. Graps of the seasonal variation of the labile carbohydrates in various mature wood sections of grapevines. (A) The three-year and older trunk; (B) the two-year and older root; (C) the one-year root; and (D) the rootlets (wood and bark).

RELATIVE UTILIZATION OF CARBOHYDRATES

In the stem bark the spring and summer utilization exhausts the stored starch (fig. 3), whereas a comparatively large amount is retained in the root bark. In aboveground parts, the carbohydrate content fluctuated most in the trunk bark (figs. 1, 2, 3). Judging from this fact, the capacity of the

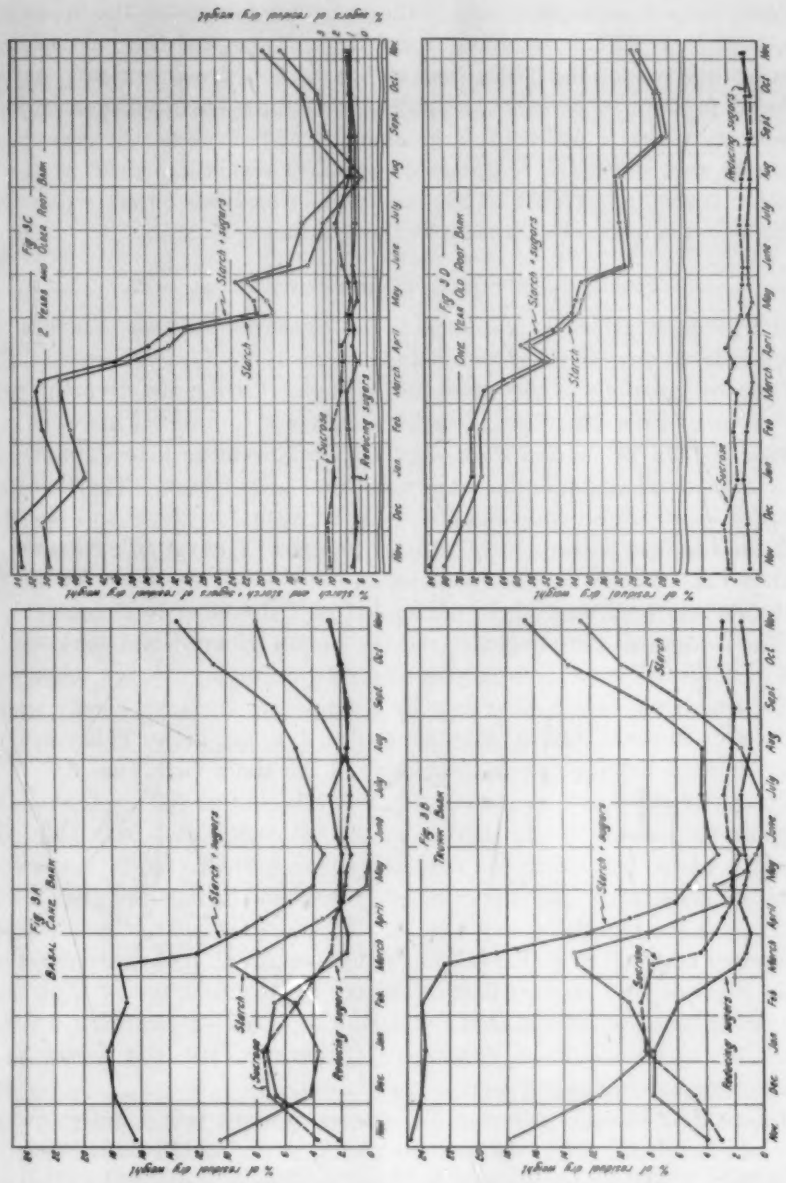

FIG. 3. Graphs of the seasonal variation of the labile carbohydrates in the bark of various mature sections of grapevines. (A) The base of the canes; (B) the trunk; (C) the two-year and older roots; and (D) the one-year-old roots.

stem bark as a storage tissue increases with age, a situation that might result from an increased ratio of parenchymatous storage cells in the thick, older bark.

In the wood, carbohydrate utilization decreases rather uniformly from

the middle cane section through the intervening sections to the inner trunk wood (figs. 1, 2A). In going from the one- to the two- to the three-year + trunk wood, the utilization of the carbohydrate content decreased from 55 to 35 to 26 per cent., respectively (figures based on maximum and minimum values). Although large amounts of carbohydrates were utilized from the root storage, a considerable quantity was still present at the late summer minimum. WIGHT's (**32**) observations on *Pinus sylvestris* also show large amounts of starch throughout the growing season.

THE LEVEL OF SUGARS DURING THE ANNUAL CYCLE

Large fluctuations of the sugars in "mature" tissues occurred only through interconversions of starch and sugars in the dormant season. Since the general relations of the starch to the sugars during the dormant period have already been discussed, it appears sufficient to note an increase in the sucrose as well as the reducing sugars from November 6 to January 8 in all aboveground parts, both in the wood and in the bark. The increase is followed by an almost equivalent decrease between January 8 and March 17. This finding is in direct contrast to the report of KRAYBILL, SULLIVAN, and MILLER (**12**) that as sucrose increases in the wood it decreases in the bark of the aboveground portions of the apple tree (November to December).

The content of reducing and sucrose sugars in wood and bark samples of the "mature" stem sections showed a slow decrease, through the summer period which was interrupted only by a temporary increase, chiefly sucrose, in the stem-bark sections in early June (figs, 1, 2, 3A, 3B). This temporary early summer increase was also evident in the shoot bark (fig. 6). These observations agree with an earlier report by WINKLER (**33**). A temporary increase also occurs in the sucrose in the two-year + root bark; but there is only a slight increase in the reducing sugar content (fig. 3C), occurring after the blossoming and major shoot growth and before the period when the fruit gains rapidly in dry weight. These increases may result from a temporary state of slow utilization, with a resultant transient increase in sugar content, and may explain MURNEEK's (**15**) findings of high sugar content in the apple tissue at the blossoming period—a condition which he attributed to hemicellulose hydrolysis. HOWLETT (**10**) also found an increase in sugars in apple flowers at full bloom.

In spring and early summer, the sucrose content was slightly lower in the basal cane bark than in either the trunk or the middle cane bark (figs. 3A, 3B). The bark of the one-year and two-year + roots had a distinctly lower sucrose content than the stem-bark sections (figs. 3C, 3D). The sucrose content of the outer trunk wood drops less rapidly than that of the cane wood in early spring, a fact which perhaps indicates a slower local utilization or a peak as a source of supply gradient (fig. 1). This condition persists only a relatively short time and then, with the increase in the sucrose content of the canes, beginning with the blossoming date (May 24), a reverse gradient appears.

There is a gradient in the decrease of the sucrose content from the outside one-year-old trunk wood to the inner three-year + wood in spring (figs. 1C, 1D, 2A). If sucrose, at least in the phloem, is conceded to be the form in which carbohydrates are transported, as indicated by the investigations

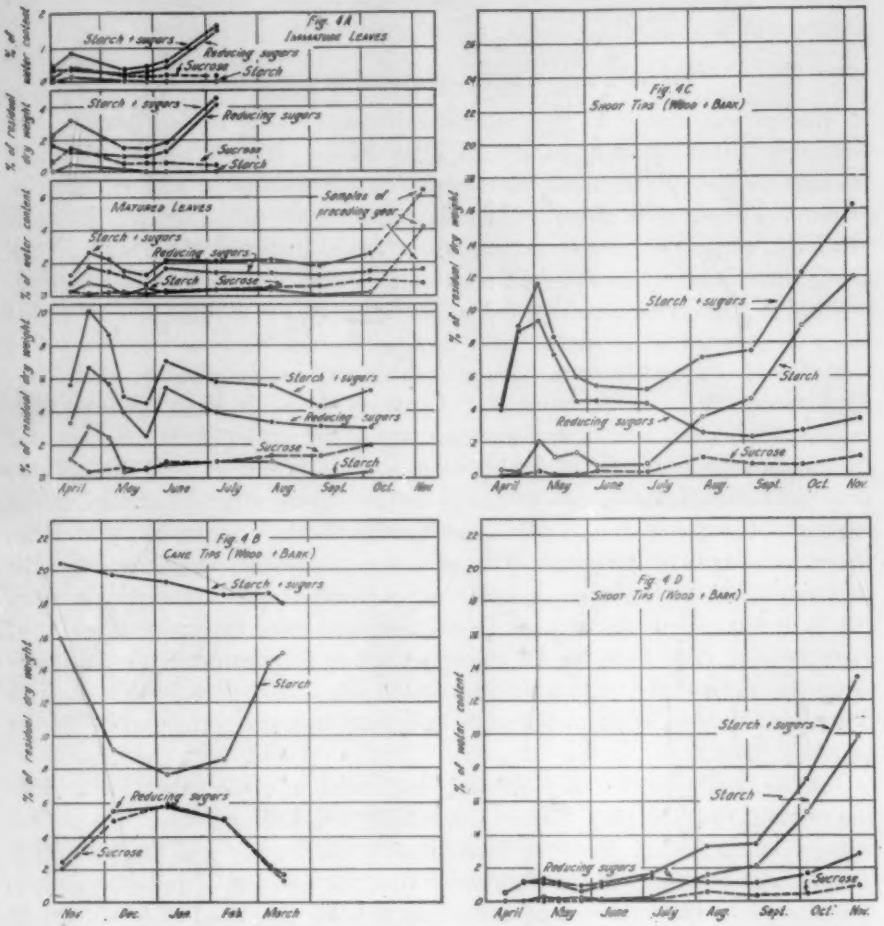

FIG. 4. Graphs of the seasonal variation of the labile carbohydrates in various sections of grapevines. (A) The immature and mature leaves expressed both as percentage of the water content and as percentage of the residual dry weight; (B) the cane tips (wood + bark) as percentage of the residual dry weight; (C) the shoot tips (wood + bark) as percentage of the residual dry weight; and (D) the shoot tips (wood + bark) as percentage of the water content.

on phloem exudate, the decreasing gradient inward might signify that the drop in total carbohydrates in the inner trunk sections is due to respirational losses.

Both the wood and the bark of root sections have a slightly higher sucrose content (figs. 2, 3) in winter than in summer. This higher content was

reduced more or less uniformly to the summer minimum. The root-bark sections and rootlets seem to show a summer minimum, although there is much irregularity in the data (figs. 2D, 3C, 3D). In spring the sugar content of the small roots showed no such decided increase as that which led BUTLER, SMITH, and CURRY (2) to suggest a movement of sugar to the above-ground stems.

STARCH AND SUGAR RELATIONS IN THE SHOOTS

Starch was absent in the immature leaves (except in one sample), as well as in the middle shoot bark, until after July 6 (figs. 4A, 6A). Although present in the other sections, the starch content fluctuated without correlation to the quantity of sugars, when expressed on a residual dry-weight basis (figs. 6A, 6C). On the percentage water basis, however, there was a relatively close correlation between sugar and starch contents (figs. 6B, 6D).

Starch storage became marked in the wood of the middle section of the shoot on July 6 (fig. 5) compared with August 6 for the basal section, a fact that may indicate a basipetal storage such as has been observed by MER (14), SWARBRICK (28), and GARDNER (7). Contrary, however, to the general findings of other observers as well as those mentioned above, starch storage did not begin first in the bark nor in the apical tips (figs. 4C, 4D, 6). These data seem reasonable in view of the probable photosynthetic function of the cortex of the green shoot and the utilization of these materials that takes place at the apex of the shoot. This situation is probably exaggerated in the properly pruned grapevine, as compared with other woody perennials, since the long, succulent shoots bear large, mature leaves throughout much of their length. The midpoint of the shoot might be considered as the maximum point of supply, with utilization occurring at either end—that is, with downward translocation to the stem and root, upward translocation to the growing point.

In the succulent tissues (leaf and shoot sections), the sugars, especially the reducing sugars, play the most significant rôles during the spring. The amount of sucrose in the shoot sections and the mature leaf samples (figs. 4, 5, 6) was relatively low as compared with the amount of reducing sugar. The sucrose of the shoot tips remained remarkably low until after the apical growth began to slow down (figs. 4C, D). The reducing sugar content, however, was relatively very high on a residual dry-weight basis in late spring and then decreased to a late-summer minimum. This condition of decrease from the high sugar content of the succulent green shoots as the season advances has been reported by numerous workers, such as HARVEY (8) and SMYTH (27) for apple varieties, SCHRADER (22) for the Concord grape, and PICKETT and COWART (17) for the muscadine grape.

Apparently, however, this general finding of a high sugar content in the succulent tissues may result largely from the basis used in expressing the data. Not much significance can be attached to comparisons of data, calculated on a dry-weight basis, for succulent tissues with data for mature,

lignified wood, in which the ratio of cell-wall substance per living cell is very much greater. The data for the carbohydrates of these tissues, based on the original water content (figs. 4, 5, 6), have been included, since this method of detecting changes in the individual cells appears to be the best available, even though it cannot be considered wholly satisfactory.

The curves of figures 4, 5, and 6 show that on the basis of water content there was, at the growing tip or in the other succulent sections, no considerable concentration of sugar such as seemed to occur in these tissues when the

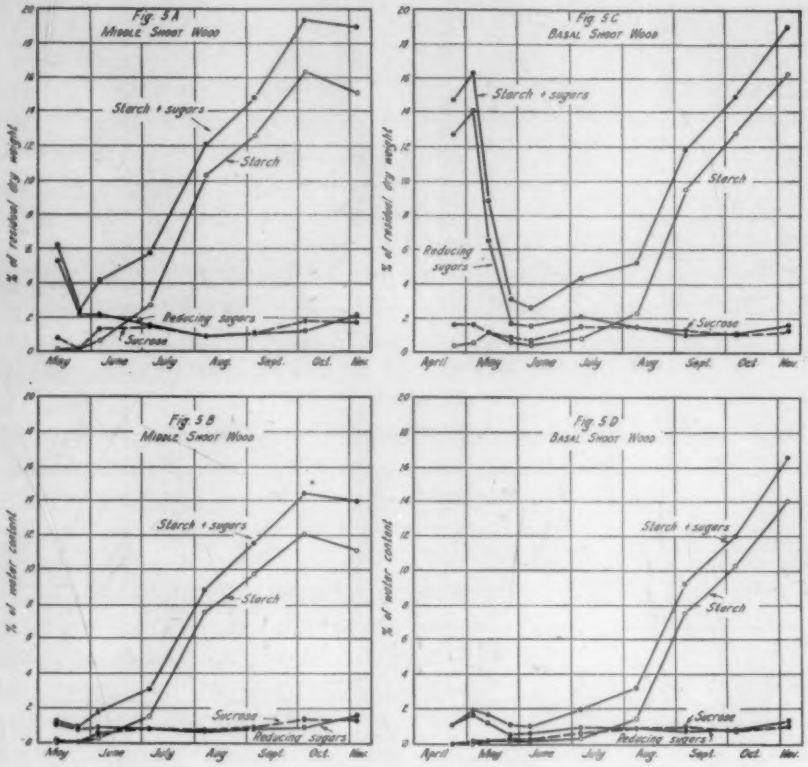

FIG. 5. Graphs of the seasonal variation of the labile carbohydrates in various wood (current season) sections of grapevines. (A) The middle of the shoots, and (C) the base of the shoots expressed as percentage of residual dry weight; and (B) the middle of the shoots, and (D) the base of the shoots expressed as percentage of water content.

results were calculated on a residual dry-weight basis. Results obtained on the former basis appear to be more reasonable, in the light of the downward gradients from leaf source to points of utilization noted by SWARBRICK (**28**) and CAMERON (**3**), together with the rapid utilization in the succulent parts. The high apparent concentration of sugar in the succulent sections, when expressed on a dry-weight basis, therefore seems to result chiefly from an unsatisfactory basis of comparison; that is, this tissue has a low-average weight of residual wall material per living cell. This viewpoint of a lower

concentration at the point of utilization is supported by the low sugar content of the rapidly expanding immature leaves. On either basis, the earliest samples of the shoot tips and of the basal shoot bark, as well as those of "mature" leaves, exhibit the lower reducing-sugar content (figs. 4, 6). This situation may result from a limited rate of carbohydrate translocation from the storage tissues, combined with rapid utilization.

The greater reducing-sugar content of the young and very succulent basal shoot wood of April 22 and May 4, either on a dry-weight basis or on

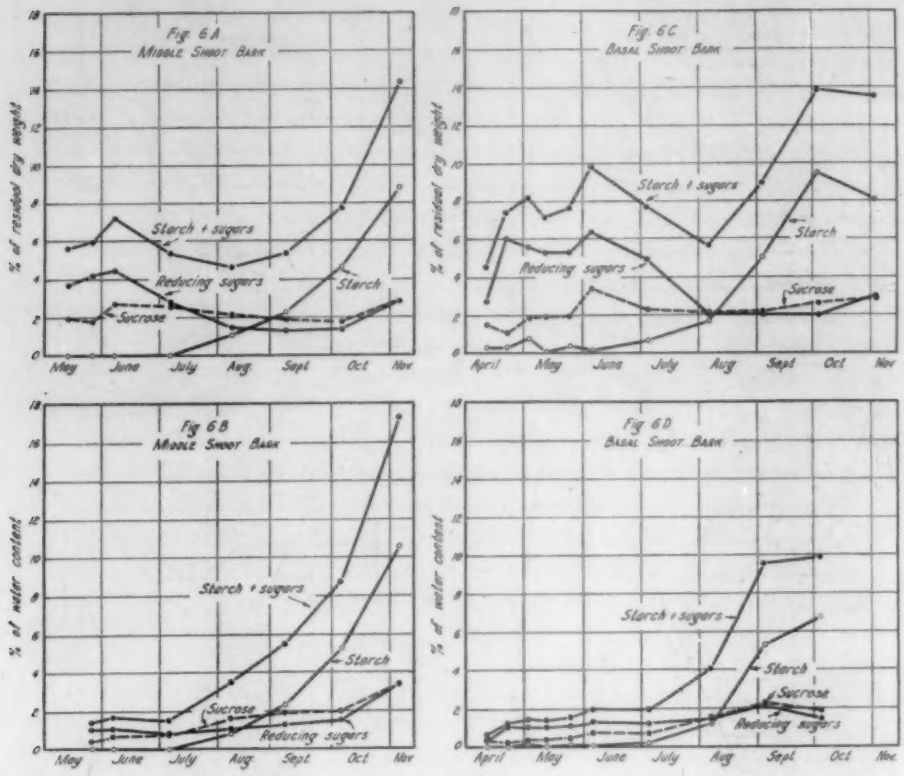

FIG. 6. Graphs of the seasonal variation of the labile carbohydrates in the bark (current season) of various sections of grapevines. (A) The middle of the shoots, and (C) the base of the shoot expressed as percentage residual dry weight; (B) the middle of the shoots; and (D) the base of the shoots expressed as percentage water content.

a percentage-water basis, as compared with the bark of the same sections (figs. 5, 6), possibly results from a more rapid enlargement of the cells of the xylem—an enlargement that temporarily gave this tissue a greater ratio of cell content to cell wall than that existing in the bark. This supposition seems to be partially confirmed by the higher water content of the wood as compared with the bark (unpublished data). The results agree with those obtained by SMYTH (27) for similar tissues of apple shoots.

The decrease in reducing sugar that occurs in the basal sections of the

shoot wood between May 4 and 26 (on percentage-water basis), as compared with that of the bark sections, which remain approximately constant, perhaps results from the retention of water by the newly differentiated tracheal elements of the xylem; such retention would have a diluting effect on the sugar content when the calculation is based on total water content (figs. 5C, 6C). If so, evidently this basis as a measure of change within the individual cells of differentiating tissues is likewise inadequate.

Summary

1. The winter disappearance of starch in Vinifera grapevines is almost quantitatively accounted for by the appearance of an equivalent amount of sugar in the above-ground sections. There is no evidence of dormant-season carbohydrate translocation, nor of the formation of hydrolysis of hemicellulosic or materials other than starch and sugars.

2. The conversion of starch to sugar produces approximately equal quantities of reducing sugars and sucrose.

3. The approximate constancy of the sugar values of comparable sections suggests control of the starch hydrolysis by the concentration of the sugars.

4. There is no evident winter conversion of starch to sugar in the roots.

5. A slight amount of growth, or at least a higher respiration rate, seems to be indicated by the more rapid rate of starch loss in the roots during the winter.

6. The decreases in carbohydrates during the dormant season indicate a respirational loss of about 0.5 per cent. per month in the wood of the stems.

7. The gradients of utilization of carbohydrates so often observed microscopically are not clearly evident, though primary utilization of bark reserves is indicated. The comparative irregularity and the relatively small percentage of utilization from the inner trunk sections seem to agree with the findings showing that only small, mostly localized areas are depleted of reserves by early shoot growth.

8. Extremely high starch storage occurs in the bark sections of the roots and in the rootlets.

9. Even at the summer minimum, relatively large amounts of stored carbohydrates were maintained in all the "mature" wood tissues.

10. Aside from the winter conversion of starch to sugar, the sugar content of the "mature" tissues did not fluctuate markedly. In these tissues, approximately equal quantities of the sucrose and reducing sugars were present throughout the year.

11. In the shoot wood there is an indication of a gradient of fall starch storage toward the apex and toward the base from a mid-point supply center.

12. The starch and sucrose in the succulent green tissues are of relatively minor importance as compared with the reducing-sugar content.

13. As a means for comparison of "mature" tissues, it is concluded that a starch- and sugar-free, dry-weight basis is probably most satisfactory. For differentiating succulent tissues, however, the water content is probably

more nearly related to the number of living cells, and hence would be the most satisfactory base for evaluating comparative changes in the cells.

UNIVERSITY OF CALIFORNIA
COLLEGE OF AGRICULTURE
DAVIS, CALIFORNIA

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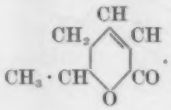
INDUCING DORMANCY IN LETTUCE SEED WITH COUMARIN

G. E. NUTILE

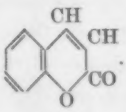
(WITH THREE FIGURES)

The inhibitive effects of extracts from ripe fruits such as tomatoes and apples on germination of seeds has been known for a number of years. It has been demonstrated that clean tomato seeds will germinate when placed on a moist substratum, but will be non-viable if the substratum is moistened with a dilute solution of tomato or apple juice (**1**). This inhibition to germination was thought to be due to fruit acids. In 1934 and 1936, KÖCKEMANN (**5, 6**) produced conclusive evidence that fruit acids were not involved, but that the inhibitory effect was due to neutral compounds soluble in alcohol and ether and relatively heat stable.

HEATON (**3, 4**), working with malt extract and yeast, first described the occurrence in plants of a factor inhibiting the growth of animal connective tissues in tissue cultures. In 1943, MEDAWAR (**10**) believed that the inhibiting compound in malt extract was probably closely related to or identical with parasorbic acid, which accompanies sorbic acid found in the berries of the mountain ash (Sorbus). This worker synthesized a small amount of parasorbic acid, and found the synthetic compound to be active. KUHN (**7**) proved convincingly that parasorbic acid is an unsaturated lactone of the structure



In 1943, KUHN, JERCHEL, MOEWUS, MÖLLER, and LETTRE (**8**) provided further evidence on the growth and germination inhibiting properties of synthetic parasorbic acid. Because of the close structural relations between parasorbic acid and coumarin, these authors also tested the latter compound and found it to be even more active in inhibiting germination of garden cress (*Lepidium sativum* L.). Coumarin has the following structural formula



It was not clear whether these inhibitions of germination were due to toxicity or actually represented a case of inducing dormancy.

For some time, bio-chemists have shown the presence of coumarin in various species of the plant kingdom. FREISE (**2**) states it is present in seeds of *Coumaruna polyphylla* (Hub.) Ducke, and *C. speciosa* Ducke. In eight types of sweet clover (*Melilotus alba* and *M. officinalis* SHUTT (**14**) found the coumarin content to vary from 0.81 to 1.08 per cent. in the leaf

and 1.23 to 1.43 per cent. in the stalk. WEHMER (17) mentions the presence of coumarin in 50 plant species including the following twelve Compositae: *Eupatorium aromaticum* L., *E. triplinerve* Vahl (*E. ayopana* Vent.), *E. dalea* Kth., *E. incarnatum* Walt., *E. africanum* Olv. et Hier, *Chrysanthemum segetum* L., *Liatris spicata* Wild., *L. odoratissima* Wild., *L. squarrulosa* Mich., *Ageratum brachystephanum* Sw. (*A. mexicanum* Sims.), *A. conyzoides* L., and *Rudbeckia speciosa* L. Coumarin was detected in *Prunus avium*, and *P. fructicosa* by LINGELSHEIM (9).

Since many studies have been made on the behavior of dormant lettuce seeds, it was thought a desirable species on which to conduct further studies of the inhibitory action of coumarin. SHUCK (12) showed that when a replicate series of dormant lettuce seeds were germinated on the same substratum, there was an accumulation of an inhibiting substance in the medium. He found that lettuce seeds placed on this substratum showed a decided reduction in percentage germination. This worker did not make any statement concerning the specific nature or chemical composition of this substance. Could it be that this inhibitor described by SHUCK might possibly be coumarin or compounds closely related to it? Certainly the possibility seemed to warrant further investigation.

Inducing dormancy experimentally

The coumarin used in these studies was a commercial synthetic product and was supplied by DR. WERNER BERGMANN of Yale University. One-year-old non-dormant lettuce seeds of the variety Black Seeded Simpson were used for these experiments. The general outline of the experiment was to treat seeds with coumarin solutions and then determine if treated seed responded in germination to various conditions in a manner similar to dormant lettuce seeds. Dormant seeds of the variety Grand Rapids were used for comparative studies. All germination tests were made at 18°–20° C. in petri dishes on three thicknesses of blotter and data recorded after 5 days.

EXPERIMENT I

As a starting point, the substrata for germination were moistened with various concentrations of coumarin solutions ranging from 1,000 to 1 p.p.m. Several tests were conducted under fluorescent light for eight hours a day and others in darkness. Figure 1 shows that the higher concentrations of coumarin (1,000 to 10 p.p.m.) were decidedly toxic. A careful examination of tests B and C exposed to light and test H and I tested in darkness shows that light tended to overcome the inhibitive effects of coumarin. A number of seeds in B and C showed evidence of germinating, although they were abnormal, due to toxicity. Toxic effects are also evident at 10 p.p.m. (tests D and J). The action of light in counteracting the inhibitive effects of coumarin is definitely shown in the light test (D) when compared to the dark test (J) in which 24 per cent. of the seeds failed to germinate. The above

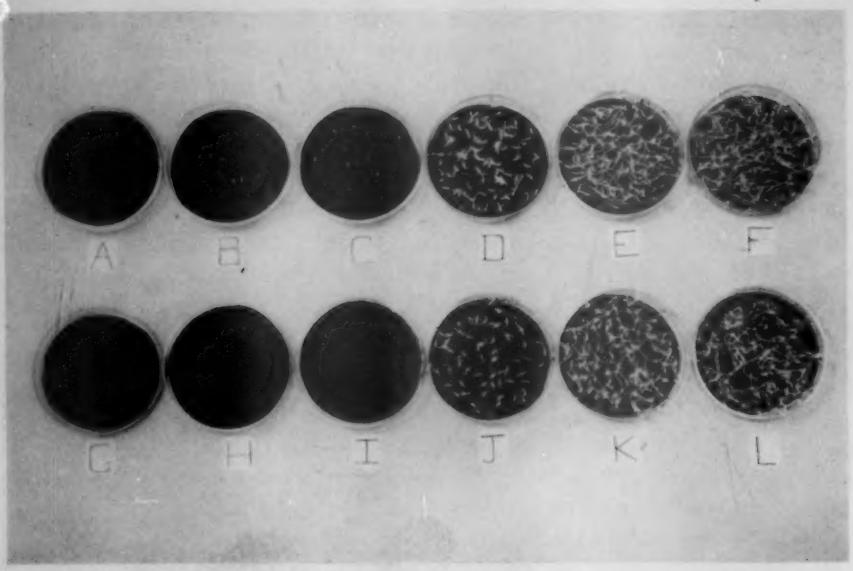


FIG. 1. Germination of non-dormant lettuce seeds on blotters moistened with various concentrations of coumarin. Top row tested in light; lower row tested in darkness. Concentrations from left to right: A and G, 1,000 p.p.m.; B and H, 100 p.p.m.; C and I, 75 p.p.m.; D and J, 10 p.p.m.; E and K, 1 p.p.m.; F and L, controls. Compare tests B and H and C and I. Note that some seeds in B and C (light tests) have attempted to germinate. Note also the ungerminated seeds in J (dark test) as compared with 100 per cent. germination in test D (light test).

experiment clearly indicated the inhibitive effect of coumarin on non-dormant lettuce and that light exhibited a tendency to overcome this inhibition of germination. By this method we were unable to obtain a concentration which would induce a so-called dormancy and not produce conditions toxic to germinating lettuce seeds.

EXPERIMENT II

Another method was tried which proved quite effective in inducing dormancy. This consisted of soaking the seeds in 12.5 and in 25 p.p.m. of

TABLE I

EFFECT OF COUMARIN ON THE GERMINATION OF NON-DORMANT LETTUCE SEEDS. ALL TREATMENTS MADE IN DARKNESS

TREATMENT	PERCENTAGE GERMINATION	
	IN DARKNESS	IN LIGHT
	%	%
A. Seeds soaked in 12.5 p.p.m. coumarin solution*	41	99
B. Seeds soaked in 25 p.p.m. coumarin solution*	25	100
C. Control. Soaked in distilled water	98	97

* Rinsed in distilled water—before testing.

of coumarin solution in darkness at 18°–20° C. for 24 hours. At the termination of the soaking period, the seeds were removed by pouring the solution and seeds into a strainer. The seeds were rinsed thoroughly by dipping the lower half of the strainer in a vessel containing distilled water for 2 to 3 minutes. It is important to mention at this point that the above processes of soaking and rinsing must be done in darkness. Coumarin is photosentitive after entering the seed and will not exhibit any inhibitive effects on germination if the seeds are soaked in the light or if light strikes the seeds while wet. After rinsing, the seeds were tested in light and darkness. The results are shown in table I and figure 2. Tests made in darkness

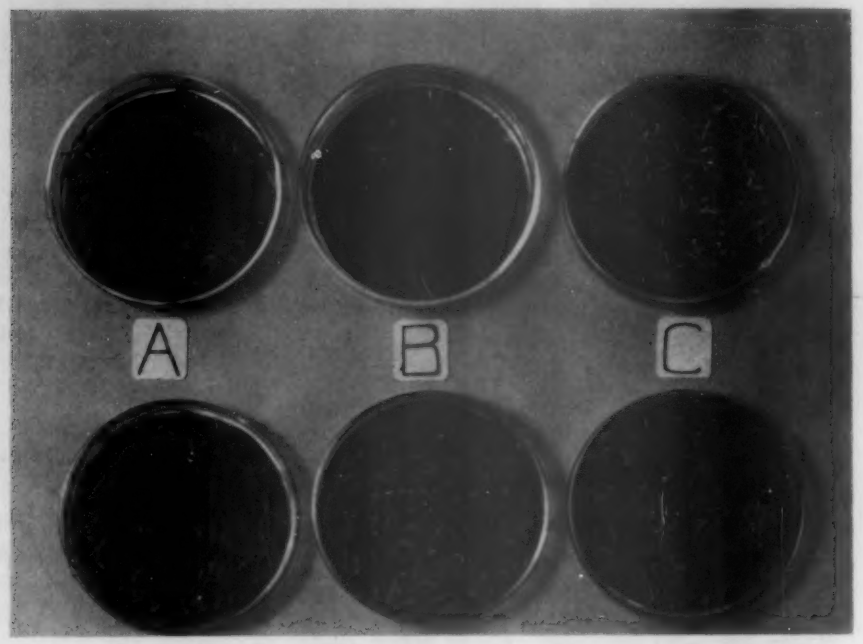


FIG. 2. Effect of coumarin on germination of non-dormant lettuce seeds. Top row tested in the dark; lower row tested under fluorescent lights. Data in table I.

revealed that dormancy was induced in a high percentage of seeds at both concentrations of 12.5 and 25 p.p.m. without causing any toxic effects; this was shown by the high germination of normal sprouts in the test germinated in the light. In other words, non-light-sensitive seed may be made light-sensitive by coumarin. It was not possible to induce this dormant condition in all seeds, since stronger concentrations of coumarin or longer periods of soaking had toxic effects.

EXPERIMENT III

Further experiments showed that dormancy could be induced in Black Seeded Simpson lettuce seeds by soaking them in distilled water in darkness and drying them in darkness. Dormancy was induced during the drying

process since it was impossible to make the seeds dormant by soaking alone. Dormancy was induced at temperatures ranging from 20° to 35° C. during the drying process. The rapidity of drying was found to be an important factor in inducing dormancy. Seeds dried slowly became more dormant than those dried rapidly at the same temperature. No satisfactory explanation can be given at present as to why dormancy is induced during the drying process following pre-soaking in water. It is well to recall at this point that dormancy was induced by soaking the seeds in 25 p.p.m. of coumarin solution without drying, and also by moistening the germination substratum with various concentrations of coumarin solutions.

Physiological responses of coumarin-treated seeds and natural, dormant seeds

Having once established the fact that this inhibitor of germination is capable of inducing a dormant condition in non-dormant lettuce seed, it was thought desirable to determine if coumarin treated seed responded in germination to various conditions in a manner similar to naturally dormant lettuce seeds.

It has been demonstrated by previous workers that prechilling and low temperatures have a tendency to break dormancy in lettuce seeds although these treatments are not as effective as light. In 1938, THOMPSON and KOSAR (**15**) reported the use of thiourea in breaking dormancy of lettuce seed. They also found (**16**) that thio compounds as ammonium thiocyanate, potassium thiocyanate, allyl thiourea, thioacetamide, and thiosemicarbazide promoted the germination of dormant lettuce seed. They offer clear evidence to show the importance of sulphur in these compounds in overcoming dormancy. Compounds similar to the thio compounds but lacking sulphur had no effect in breaking the dormant condition. The presence of sulphur, however, is not the only element necessary since they showed that ammonium sulphate, calcium sulphate, potassium sulphate, and sulphuric acid failed to promote the germination of dormant lettuce seeds. In the experiments with thiourea presented in this paper, a somewhat similar method as described by RALEIGH (**11**) was followed. The seeds were soaked in 0.5 per cent. thiourea solution in darkness at 18°–20° C., rinsed, dried 4 days in darkness at room temperature, and tested in darkness. SHUCK (**13**) working with Grand Rapids lettuce seeds showed that if dormant seeds were tested in darkness for several days, many failed to germinate when later transferred to light. In other words, when light is not supplied during the first few days after the seeds are placed in test, they go into a deeper condition of dormancy. In these present experiments, however, it was found that if the substratum was moistened with 0.3 per cent. KNO_3 after being removed from the dark and then tested in light, all seeds germinated. Similar results were obtained if the seeds were prechilled three days at 4° C. before testing in light. If KNO_3 was added to the medium in the dark and the tests extended several days in darkness, the ungerminated seed still

TABLE II

EFFECT OF THIOUREA ON GERMINATION OF NATURAL, DORMANT AND COUMARIN-TREATED LETTUCE SEEDS. ALL OPERATIONS AND GERMINATION TESTS MADE IN DARKNESS

SAMPLE	GERMINATION
	%
A. Natural, dormant seed treated with 5% thiourea for 24 hours*	83
B. Coumarin-treated seed treated with thiourea*	91
C. Natural, dormant seed; control test soaked in distilled water	2
D. Coumarin-treated seed; control test soaked in distilled water*	22

* Rinsed in distilled water.

failed to germinate. KNO_3 was ineffective in breaking dormancy if the substratum was moistened with the compound at the start of the dark test. Similar results were obtained using soil as a substratum. The effect of soil in breaking this so-called deeper dormancy in the presence of light is attributed to soil nitrates. It thus appears that KNO_3 is capable of breaking dormancy only in the presence of light.

EXPERIMENT IV

Further experiments were conducted which proved that Black Seeded Simpson lettuce seeds made light sensitive or dormant with coumarin responded to prechilling, and effects of thiourea; they then went into the deeper dormancy which could be overcome by the action of KNO_3 or soil in the presence of light in a manner somewhat similar to naturally dormant Grand Rapids lettuce seed. The results of these experiments are shown in tables II and III and figure 3.

TABLE III

GERMINATION OF NATURAL DORMANT AND COUMARIN-TREATED UNGERMINATED LETTUCE SEEDS REMOVED FROM DARK TEST AFTER 5 DAYS AND TESTED UNDER CONDITIONS SHOWN BELOW

KIND OF TEST	GERMINATION	
	NATURAL, DORMANT	COUMARIN-TREATED
	%	%
A. Tested in light on original substratum re-moistened with distilled H_2O	52	54
B. Tested in light, original substratum moistened with 0.3% KNO_3 sol. after removing from dark	96	100
C. Tested top of soil, transferred to light	96	87
D. Test continued in darkness, re-moistened with distilled H_2O	4	12
E. Test continued in darkness, substratum moistened with 0.3% KNO_3 sol.	4	15
F. Tested top of soil, test continued in dark	2	....
G. Prechilled 3 days, tested in light on original substratum, re-moistened with distilled H_2O	....	96

At present it is not known what chemical or physical reactions are involved by the use of KNO_3, in the presence of light, on the inhibitors of germination in the deeper dormant condition of natural dormant and coumarin-treated seeds. It is possible that KNO_3 may act as an oxidizing agent in the presence of light resulting in a decomposition of these inhibiting

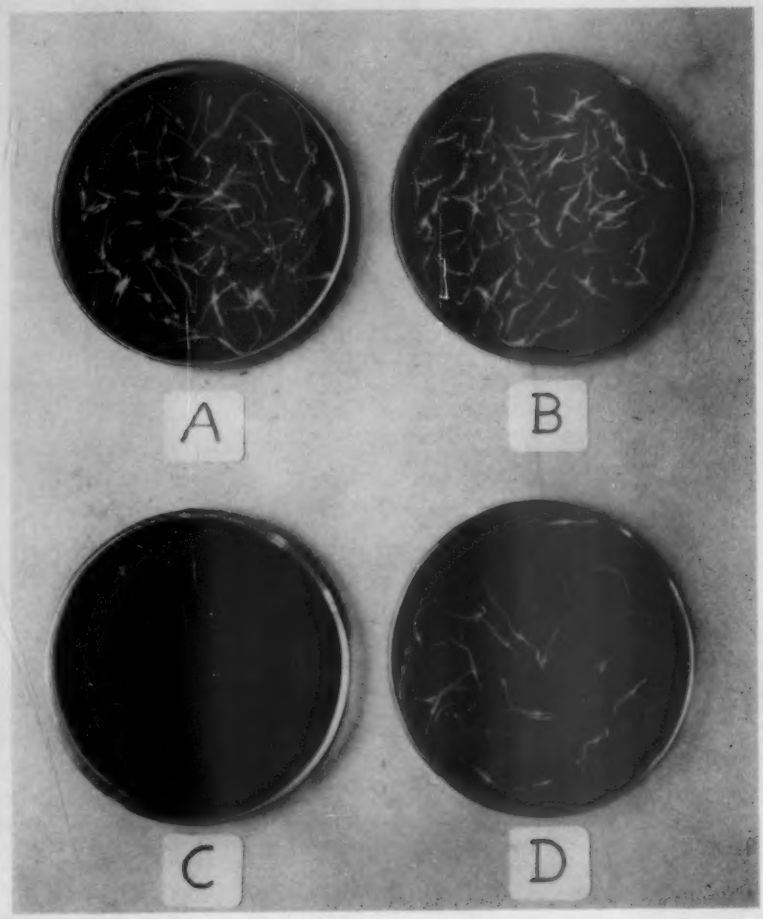


FIG. 3. Effect of thiourea on germination of natural, dormant and coumarin-treated seeds. See table III.

compounds. Just how thiourea affects these inhibiting compounds is not known at present. It is quite possible that thiourea reacts with them in some way to form entirely new compounds which possess no inhibiting qualities.

EXPERIMENT V

Results of previous experiments showed that natural dormancy in Grand Rapids lettuce seed could be overcome by exposing the seeds to high humidity and light for 24 hours. The inhibiting effects of coumarin in inducing

dormancy in non-dormant lettuce seeds has been shown. Other experiments were conducted to determine whether these processes are reversible. Could Grand Rapids lettuce seed made non-dormant be thrown back into dormancy with coumarin? Likewise, could Black Seeded Simpson lettuce seed made dormant by coumarin again be made non-dormant and again thrown back into dormancy with coumarin? It was found that Grand Rapids lettuce seed made non-dormant as described above could be made dormant by the coumarin treatment. The effects of coumarin could be overcome by exposing

TABLE IV*

DATA SHOWING THAT THE REACTIONS OF COUMARIN AND LIGHT ON THE GERMINATION OF LETTUCE SEED CAN BE MADE REVERSIBLE. WHERE INDICATED, SEEDS KEPT IN HUMIDITY CHAMBER UNDER CONTINUOUS LIGHT FOR 24 HOURS

TREATMENT	GERMINATION
	RESULTS WITH NATURALLY DORMANT SEED
	%
Naturally dormant seed (4% germ.) made non-dormant by light and high humidity	99
After making seed non-dormant and then treating with coumarin	31
Coumarin-treated seed exposed to light and high humidity	84
	RESULTS WITH SEED MADE DORMANT WITH COUMARIN
Before treatment with coumarin	97†
After treatment with coumarin	19
After exposing coumarin-treated seeds to light and high humidity	93
After exposing coumarin-treated seeds to light and high humidity and then treating again with coumarin	45

* All germination tests made in darkness. At the completion of each test any ungerminated seeds were prechilled and tested in light. Practically all seed germinated showing that the respective treatment had not destroyed the viability of the seeds.

† Germinated in darkness.

the seeds to high humidity and light and then again thrown back into dormancy with coumarin. Experiments also showed that the dormant condition of Black Seeded Simpson lettuce brought on by coumarin could be broken by exposing the seeds to high humidity and light. The seeds could then be made dormant again with coumarin. The results presented in table IV offer clear evidence that these processes are to a large degree reversible.

Discussion

The evidence does not seem yet sufficient to prove that coumarin is the inhibiting factor in natural dormant lettuce seed. It is not definitely known whether coumarin retains its chemical structure after entering the lettuce seed. It is quite possible, however, that coumarin, or its derivatives or

compounds closely related to coumarin, is the inhibitor in dormant lettuce seeds.

The effects of coumarin on varieties of lettuce seed other than Black Seeded Simpson and Grand Rapids is not known at present. Since various varieties are likely to react quite differently to similar environmental conditions during the germination process, various concentrations of coumarin solutions may be required to induce dormancy in various strains of lettuce seeds. Attempts will be made to extract and identify the inhibiting substance in dormant lettuce seed. Studies along similar lines should also be made with other species of seeds. Once the identity of these inhibitors of germination is established, we can then proceed to look deeper and more intelligently into this interesting and fascinating phenomenon called dormancy.

Summary

1. Non-dormant lettuce seeds of the variety Black Seeded Simpson were made dormant or light sensitive by soaking the seeds in 25 p.p.m. of coumarin solution for 24 hours at 18°–20° C. After soaking, the seeds were rinsed in distilled water and germinated on blotters in petri dishes in light and darkness at 18°–20° C. Coumarin is photosensitive after entering the seed and will not exhibit any inhibitive effects on germination if the seeds are soaked in the light or if light strikes the seeds while wet. Dormancy could be induced in approximately 80 per cent. of the seeds as was shown in a dark test. A germination of approximately 99 per cent. was obtained in light.

2. Coumarin-treated seed responded in germination to various conditions in a manner similar to naturally dormant lettuce seed; inhibitive effects of coumarin were overcome by light during the germination test, responded to prechilling and to treatment with thiourea.

3. Experiments showed that the processes of making dormant seed non-dormant by exposure to light and high humidity, and of inducing dormancy in non-dormant seeds with coumarin, are to a large degree reversible.

4. The evidence does not seem yet sufficient to prove that coumarin is the inhibiting factor in naturally dormant lettuce seed or if it is produced in non-dormant lettuce seeds which fail to germinate at the high temperatures of 30°–35° C. It is not known whether coumarin retains its chemical structure after entering the seed. It is quite possible that coumarin or its derivatives or compounds closely related to it is the inhibiting factor in lettuce seeds.

The author expresses his appreciation to Dr. Werner Bergmann, Associate Professor of Organic Chemistry, and to Dr. Paul Burkholder, Professor of Plant Physiology, Yale University, for their helpful suggestions and assistance during the progress of the work.

Department of Research
Associated Seed Growers, Inc.
New Haven, Connecticut

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A SIMPLE AND EFFECTIVE HUMIDITY CONTROL

R. H. WALLACE AND R. J. BUSHNELL

(WITH TWO FIGURES)

It is frequently necessary in physiological work to control the humidity of an environment in which studies are being made. Many methods of control have been devised and used. In most cases these controls are of limited application. We have recently devised an effective control which seems to have broad applications and employs simple and relatively inexpensive materials.

The device consists essentially of a radio tube whose grid is activated by the opening and closing of the contacts of a humidistat. This tube runs a motor which turns an eccentric. The eccentric compresses and releases the rubber bulb of a Bunsen water pump which delivers water from a reservoir into a glass tube leading into the top of an evaporator. The fan in the evaporator runs continuously but water to maintain humidity is added only when the humidistat calls for it. The mechanism functions very consistently and any humidity can be maintained within one per cent.

Description of apparatus

MOTOR

The most crucial unit of the control device is the motor (fig. 1, no. 3) which is a small, compact induction motor with starting fields requiring less than 50 mils for vigorous activation. This is the same motor used in the recording potentiometer and voltage regulator described in previous papers (**1, 2**). The motor has built within its case a reduction gear of 450 to 1, which gives about 3 r.p.m. on the power shaft. Since the motor activates on such low current, one can use a cheap 2A4G radio tube very successfully. Likewise, since the gear reduction is so high, there is a powerful torque delivered by the power shaft. The eccentric is obtained by mounting on this shaft a three inch wheel. Near the rim of this wheel are mounted one or more, usually two, three-quarter-inch wheels which serve as eccentrics to compress and release the rubber bulb of the water pump as the larger wheel turns in response to the activation arising at the humidistat.

WATER PUMP

The water pump (fig. 1, no. 4) used in this device works very well and can be made up in any size from parts available in any laboratory. It consists of one main glass part having three openings. Over one of these is slipped an ordinary rubber eye-dropper pipette bulb and over the other small opening is slipped an ordinary Bunsen valve. The one large opening of the glass part is fitted with a one-hole rubber stopper and glass tube. The upper end of this glass tube has a second Bunsen valve. This water pump

stands with its lower end in a water reservoir and its delivery tube in another tube leading down to the evaporator.

It functions as follows: As the larger wheel turns the small eccentrics roll accross the bulb which compresses the system and causes the delivery tube Bunsen valve to open; when the eccentric rolls beyond the bulb the natural resiliency of the rubber exerts a suction on the Bunsen valve and it opens. Thus, each time the eccentric rolls across the bulb, water is delivered to the evaporator and an equal volume is drawn in from the reservoir. In practice we have found that about one-half cc. per compression is a good delivery rate. In some cases we have used one eccentric but for high humidities usually two work better. If desirable, additional eccentrics can be added. By variation in size of the eccentric and size of bulb one can get any delivery rate that is needed.

Evaporator

A number of methods were tried for the evaporation of the water delivered. Without doubt the evaporator is the weakest unit in most controls. It must be designed to care for the particular requirements of the chamber being used. The one illustrated here (fig. 1, no. 5) is ten inches high and six inches across and it was quite adequate for a chamber thirty inches cube, even when humidities of 90 per cent. and above were being maintained at temperatures of 60° C.

The evaporator is made from a metal cylinder lined with filter paper and closed at the upper end with gauze. Inside this shell, facing upward, is mounted a small fan. We have found that an automobile wind-shield fan works quite well for this purpose. A large hole is cut at the base as an air intake.

The evaporator functions as follows: The water delivered from the pump runs down, hitting the blades of the fan and is spattered over the filter paper and gauze surfaces. Since the fan runs continuously these surfaces are essentially dry most of the time. When the humidistat calls for it, water is delivered and the fan evaporates it to bring up the humidity to the required value.

Humidistat

Any type of humidistat can be used with this device. The one which we have used and which is shown in the figure (fig. 1, no. 1) seems to be superior to most types generally used. It consists of a capillary tube of glass sealed into the end of a cellulose acetate bag. In general form it looks something like a tooth paste tube with a capillary in the top. These humidistats are made of number 7/1000 film, sealed to make the tube by means of acetone. With a little skill and patience anyone can make them. Any sensitivity can be obtained by the correct relationship between bag size and capillary size. One contact wire must pass through the point of seal between acetate and glass. A second contact is pushed down from the top of the capillary. The bag and part of the capillary are filled with mercury. Since the bag is

collapsible, it can be filled by compressing and releasing it to drive out the air and to allow mercury to enter.

The humidistat functions as follows: Cellulose acetate expands with increased humidity. This causes the mercury column to fall back and con-

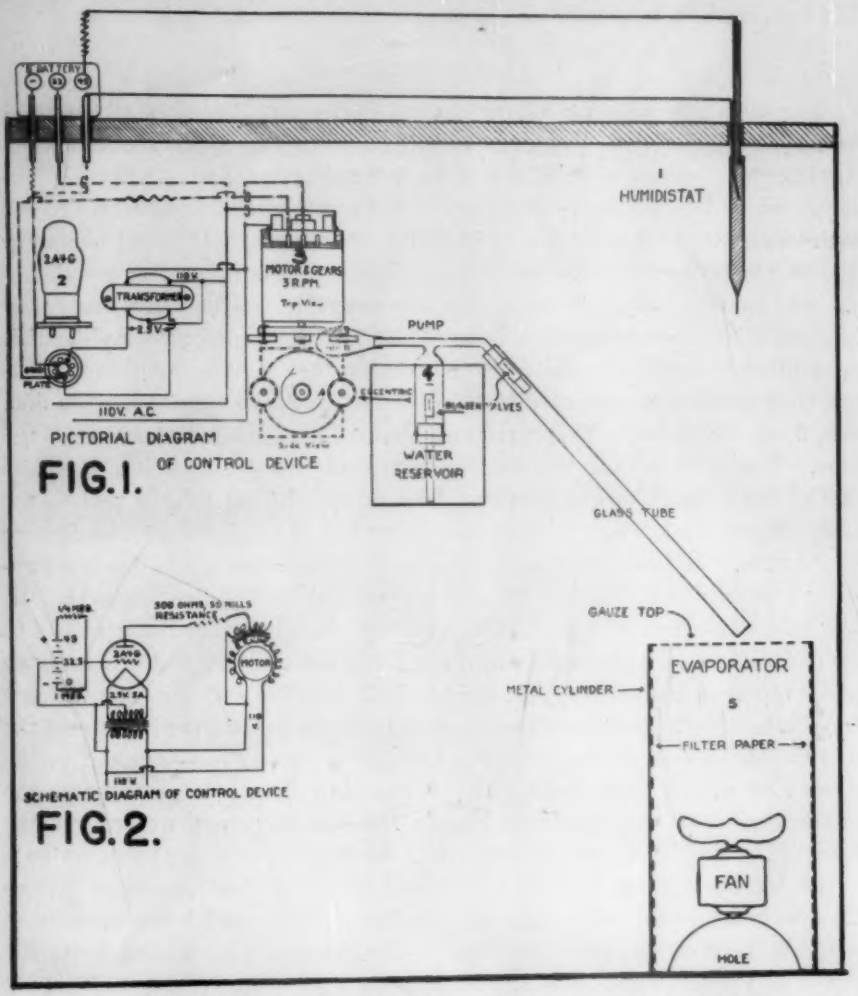

FIG. 1. Pictorial diagram showing shape and arrangement of parts as mounted inside a thirty-inch-cube environmental chamber. All wiring is likewise shown.

FIG. 2. Schematic diagram of control circuit.

tact is broken. As the chamber dries out, the bag shrinks and the mercury column rises to again make contact. The humidistat will maintain any particular set for an indefinite period of time irrespective of the intervening humidites to which it is exposed. Each humidistat must be calibrated against some standard. It was found that the ordinary hair hygrometer was highly unreliable when either limit of the scale was approached. Like-

wise, temperatures above 45° C. rendered that instrument unusable. Many checks between the Fries hygrometer and a wet-dry junction thermocouple connected to a recording potentiometer were found to agree rather well, usually within 1–5 per cent. for general biological ranges of temperatures. The humidistat is nearly linear for temperatures from 15° to 45° C.

The electrical circuit

The schematic diagram of the control device (fig. 2) gives all essential details of the electrical circuits. It will be noted that there are four principal parts: a motor, a thyratron tube, a transformer, and a battery. The motor which has been mentioned above is a small compact induction motor with two built-in transformers as starting mechanisms. It is not necessary that this motor be reversible but it costs little more than a similar one that is not and has the advantage of giving one complete freedom in direction of rotation of the eccentrics which is frequently advantageous for simplification in building. It will be noted in the figure that the common junction of these two transformers is connected to the plate of the 2A4G tube through a 500-ohm fixed resistance. This resistance prevents possible overloading of the tube. The other lead to the starting transformer being used is tied to one side of the 110-volt power supply. The cathode of the tube is tied to the other side of the 110 line through the center tap of the filament transformer of the tube. If there were no further wiring, the tube would fire continuously because the 2A4G tube fires on a zero grid bias or floating grid. If, now, one connects a 45-volt B battery in the circuit so that the grid ties to the minus through a grid leak and the 22½-volt tap connects to the center tap of the filament transformer, the grid is held negative and the tube will not fire at all. The final connections necessary for the control are to connect the grid to one wire of the humidistat and connect the 45-volt terminal to the other wire of the humidistat. The device now functions as follows: the mercury rises in the capillary, closing the contact which neutralizes the minus-22½ grid bias on the tube and the tube fires. The motor runs, water is added, humidity rises, and the humidistat breaks contact causing a minus-22½ grid bias and the tube again ceases to fire. The ¼- and 1-meg resistances in the battery circuit give shelf life to the battery. All wiring from the 45-volt tap to the humidistat and back to the grid must be very thoroughly insulated or the device will not function. It is possible to eliminate the B battery by using a small 6H6 rectifier tube in its place. This is scarcely worth while, however, because a small inexpensive B battery will last for at least two years.

This humidity control has functioned very consistently for long periods of time. It can be adapted to fit almost any conditions. A recording hygrometer placed in the chamber gives straight lines for weeks at a time. Tests with a wet and dry junction thermocouple on a recording potentiometer have shown, however, that there are small fluctuations of around

0.25 per cent. These appear on the record as almost mathematical undulations every five to ten minutes in our particular chamber.

DEPARTMENTS OF BOTANY AND ZOOLOGY
UNIVERSITY OF CONNECTICUT
STORRS, CONNECTICUT

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THE RESPIRATION OF GROWING PLANT CELLS

WINIFRED DONNELLY AND W. A. BECK

(WITH ONE FIGURE)

Previous investigations have shown that young, growing plant cells exhibit certain phenomena which apparently depend directly upon the metabolic activity of the protoplasm (**1, 2, 3, 4, 5, 6**). In the present study, an effort has been made to determine changes in the metabolic activity of such cells, using the rate of respiration as an indicator.

Methods

Growing cells of the hypocotyl of Helianthus seedlings were used, employing conditions identical with those of previous experiments cited above. The uppermost thirty-five millimeters of hypocotyl were cut in seven segments of uniform length (5 mm.). The region in which the cells were embryonic was not more than one millimeter long. At a greater distance than two millimeters there were no embryonic cells but all were in the stage of elongation. In the seventh or basal zone all cells were mature. The respiration of each of the whole five-millimeter sections was determined. In a series of ten supplemental experiments, the respiration of the uppermost two-millimeter section was measured in order to determine possible differences in the respiratory rate of embryonic and elongating cells.

In some series of experiments the sections were taken from the same hypocotyl. In such cases the hypocotyl was either left on the rootstock or thirty-five millimeters of it were cut away at once and left lying in a moist chamber. When it was left on the rootstock the zones were cut away successively beginning at the tip. Otherwise the zones were cut away successively beginning below. The respiration of each zone was determined in direct succession. In other series of experiments only one section was taken from any one hypocotyl. By this combination of procedures an effort was made to compensate for possible errors that might result from the injury done to the hypocotyl by sectioning.

A modification of the differential micro-respirometer previously described was used (**8**). The modification consisted in sealing a graduated capillary to the "experimental chamber." The pressure was kept constant by raising a mercury column into the graduated capillary which, by special arrangement, could be levelled with ease. The respirometer has these distinct advantages: it occupies little space; its temperature does not readily change in the constant temperature water bath; it has few separable parts; it is relatively easy to manipulate; and it is highly sensitive. Not more than two minutes elapsed from excision and first reading to verify equilibrium. A period of fifteen minutes was allowed for the establishment of equilibrium, after which records were made.

Results and discussion

The number of cubic millimeters of oxygen at 0° C. and at 760 mm. pressure were determined and listed in table I. The figures are the averages of more than 20 separate determinations.

Every section consumed less oxygen as time progressed, probably as a result of the exhaustion of the food materials which are no longer supplied from the cotyledons. It is also evident that in general the younger zones respire more vigorously than the older zones. The relatively high rate of respiration in the third zone is remarkable; one might have expected it to be lower than that of zones one and two, since the cells of zone three were older. The lower rate of respiration of the fourth and older zones may be due to one of two causes or a combination of both; i.e., the relative age of the

TABLE I

CUMULATIVE OXYGEN CONSUMPTION IN CUBIC MILLIMETERS BY DIFFERENT ZONES OF THE HYPOCOTYL

TIME IN MINUTES	ZONES (EACH 5 MM. LONG)						
	I	II	III	IV	V	VI	VII
min.	*mm.*³	*mm.*³	*mm.*³	*mm.*³	*mm.*³	*mm.*³	*mm.*³
5	4.38	2.29	2.19	1.07	0.36	0.12	0.07
10	6.42	3.90	3.58	1.32	0.36	0.22	0.12
15	9.18	4.33	3.69	1.47	0.36	0.31	0.15
20	10.72	4.94	3.97	1.69	0.45	0.31	0.15
25	12.97	5.42	4.74	1.82	0.45	0.31	0.18
30	13.65	5.93	5.45	2.01	0.45	0.33	0.19
35	16.16	6.57	5.62	2.19	0.61	0.34	0.20
40	17.88	7.39	5.77	2.34	0.61	0.34	0.26
45	18.91	8.07	6.31	2.36	0.61	0.35	0.26
50	19.84	9.03	7.08	2.63	0.66	0.35	0.27
55	20.76	9.05	7.77	2.70	0.66	0.36	0.29
60	20.87	9.05	8.18	2.92	0.66	0.36	0.29

protoplasm or to the relatively low number of cells per unit volume of tissue in older zones. In order to determine the effect of age on the protoplasm, an approximate calculation of the relative number of milligrams of oxygen consumed by the unit mass of protoplasm in each zone was made. The consumption of oxygen was not based on the unit mass of wet weight or dry weight because the cell wall and the cell sap do not respire.

Since the mass of the protoplasm does not increase appreciably during the process of cell enlargement the total mass of protoplasm in any zone is directly proportional to the number of cells present. An approximation of the relative number is not difficult, since the diameter of the cells remains almost constant during the process of cell enlargement. By actual measurement the average diameter was found to be 46 microns in embryonic cells and 47 microns in mature cells of the seventh zone. The number of cells in a zone is directly proportional to the number of cells in its cross-section and the number of cells in its five-millimeter length. The number of cells in the cross-section is constant (because the diameter of the cells

remains constant) and the number of cells in the five-millimeter length is inversely proportional to the average length of the cells. The average length of the cells and the relative number of cells for each zone are listed in table II.

The quantity of oxygen consumed by any zone divided by the number of cells in that zone expresses the oxygen consumption per unit (arbitrary) mass of protoplasm. These values give adequate expression to the rate of respiration of the protoplasm in the different zones, so that they become comparable and indicate the change in the rate of respiration correlated with the aging of the protoplasm.

The values thus calculated are presented in the form of graphs (fig. 1). To discover whether the greater rate of respiration in Zone I was connected with cell division, sections only two millimeters long, directly below the base of the cotyledons were used in supplemental experiments. The values

TABLE II

THE AVERAGE LENGTH OF THE CELLS IN MICRONS AND THE RELATIVE NUMBER PRESENT IN EACH ZONE

ZONES	I	II	III	IV	V	VI	VII
	μ	μ	μ	μ	μ	μ	μ
Average length	48	81	116	145	168	190	208
Number	208	123	86	68	59	53	48

obtained from these experiments are depicted as graphs. It is apparent that the rate of respiration in the proliferation zone far exceeds that of the rest of Zone I, where cell enlargement has already begun. This is no doubt due to the process of mitotic division because OSTERHOUT (9) showed that respiration is much more rapid in the nucleus than in the cytoplasm. It is also apparent that the great differences shown in the respiration of the different zones (table I) are largely due to the greater amount of protoplasm present in the younger zones. Nevertheless, even if allowance is made for this factor, younger protoplasm respires more vigorously than older protoplasm. Zones I and III both have unusually high rates of respiration and it is particularly remarkable that the respiratory rate of the protoplasm in Zone III is greater than the respiratory rate of the protoplasm in Zone II. It is furthermore remarkable that for the first ten minutes the respiration rate of Zone III is greater even than the respiration rate of protoplasm of Zone I. This is not accidental since the average of the numerous experiments is in accord with all of the individual experimental tests.

The number of milligrams of oxygen consumed per unit mass of protoplasm in the region of proliferation of cells is very much greater than that in the third zone at any time. It still remains true, however, that the respiration per unit mass of protoplasm is augmented in the third zone after a steady decline occurred in the first and the second zones. This fact should be correlated with the other growth phenomena previously reported and

described by BECK and ANDRUS (2): "The protoplast and the osmotic quantities (in Zone III) change according to the described gradients. The rigidity of the cell walls is negligible but there are indications of a definite increment in the rigidity, which comes to clear expression in the fourth zone,

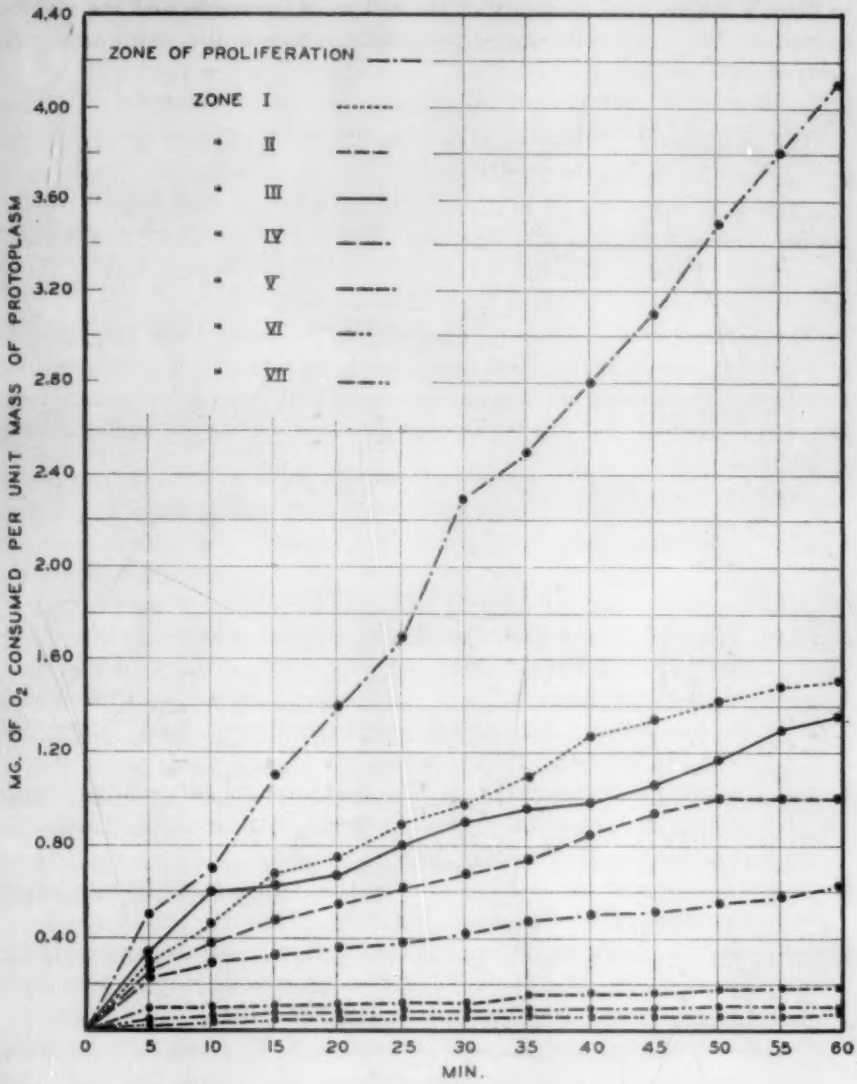

FIG. 1. Respiration rate of growing cells of the hypocotyl of sunflower seedlings.

where it manifests its influence on the wall pressure and the suction tension." It appears evident that the respiratory rate increased considerably in the third zone because some fundamental stimulation of the metabolic activity occurred. We are inclined to think that this is probably due to the influence of auxin. This view is supported by the findings of BONNER (7) that elongation does not take place in the absence of oxygen; that the

rate of respiration is increased by the addition of growth substance and inhibited by KCN; and that increase in respiration due to growth substance is essential. It is also remarkable that the respiratory rate of the mature cells is relatively low and almost negligible. The decline occurs suddenly in Zone V where "the elasticity of the cell walls decreases and the rigidity increases" (**2**). The geotropic response in this zone is also slight or at least delayed (**3**).

Summary

The respiration of the growing cells of the hypocotyl of *Helianthus annuus* was determined with the aid of a micro-respirometer. The rate of respiration is expressed in milligrams of oxygen per unit mass of protoplasm. The rate declines with time for every section, no doubt as the result of the excised state. The highest rate of respiration occurs in the uppermost two millimeters in which the embryonic cells occur.

The average rate declines successively in the zones from the youngest to the oldest except in the third zone where the rate of cell enlargement is maximum. The respiration rate was correlated with other growth phenomena and it is suggested that auxin exercises an effect on the metabolic activity of the protoplasm.

INSTITUTUM DIVI THOMAE AND
OUR LADY OF CINCINNATI COLLEGE
CINCINNATI, OHIO

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NOTE ON GALL FORMATION IN DECAPITATED YOUNG BEAN PLANTS

LEWIS H. FLINT AND CHARLES F. MORELAND

(WITH ONE FIGURE)

In the course of a series of experiments with young bean plants under continuous irradiation previously described in this journal (1), an accessory set of trials was carried out in the greenhouse. Although the results obtained appeared to have little significance in relation to quality of light, an unexpected and somewhat striking development of galls took place. Subsequently a modified second series of similar greenhouse experiments was carried out. The results obtained in the two sets of experiments seemed sufficiently interesting and suggestive to warrant record.

Materials and methods

In both sets of experiments Black Valentine beans were started in six-inch pots of sand and loam on a bench in a greenhouse. Shortly after emergence the plants were thinned to five per pot. When the compound leaves had begun to unfold, the desired number of pots of plants was selected for uniformity and all plants were decapitated just above the axils of the simple leaves.

In the first series of experiments, four pots of plants were put into each of three moist chambers covered with cellophane while four pots of plants were left alongside on the greenhouse bench. Aside from occasional watering, no further treatment was given. In the second series of experiments, a similar procedure was followed, but some treatments designed to induce gall formation were added. In one of these treatments the cut ends of the plants of four pots were covered with 4 per cent. indole-acetic acid in lanolin; they were then placed in humid chambers. In another of these treatments the plants of four pots were treated with smears of *Phytomonas tumefaciens* from an agar culture before being placed in humid chambers.

Special attention was given to the plants of the remaining two sets of four pots each. Previous to decapitation the appropriate stem portions were swabbed with cotton dipped in 95 per cent. alcohol. Cutting was done with a scalpel from 95 per cent. alcohol and as the decapitation took place, a small piece of sterile cotton was placed quickly over the cut end with sterile forceps and tamped lightly around it. Four pots of plants thus treated were placed in humid chambers to constitute one set while four other pots of plants treated in the same manner were left on the greenhouse bench.

In all cases the humid chambers consisted of 12-inch glazed pots containing an inch of water. Wire frames were superimposed upon these glazed pots and green or red cellophane sheets were then wrapped over the

TABLE I

RESPONSES OF DECAPITATED YOUNG BEAN PLANTS TO VARIOUS TREATMENTS IN A GREENHOUSE

TREATMENT AND CONDITIONS	PLANT RESPONSE
Humid chamber covered with red cellophane	Frequent gall formation, including discoid, efflorescent galls
Humid chamber covered with green cellophane	Frequent gall formation, including discoid, efflorescent galls
Humid chamber covered with clear cellophane	Frequent gall formation, including discoid, efflorescent galls
Open bench in greenhouse	Cut terminals withered, brown. No galls of any kind

frames and held to the outside of the pots with gummed paper. There were thus provided two pots with red cellophane and two with green cellophane for three of the indicated conditions. All plants remained on the greenhouse bench during the experiment.

Results

The results obtained have been brought together in tables I and II. Galls developed in the humid chambers but under no other condition. The type of gall which developed has been indicated in figure 1. Following

TABLE II

RESULTS OF VARIOUS TREATMENTS ON THE DEVELOPMENT OF GALLS IN DECAPITATED YOUNG BEAN PLANTS IN A GREENHOUSE

TREATMENT AND CONDITIONS			PLANT RESPONSES		
			EPINASTY*	BUD INHIBITION	GALLS†
Growth substance: indole-acetic acid in lanolin	Humid chamber	Red cellophane	+ +	+ +	+ +
	Humid chamber	Green cellophane	+ +	+ +	+ +
Bacteria: *Phytomonas tumefaciens* in agar	Humid chamber	Red cellophane	− −	− −	+ +
	Humid chamber	Green cellophane	− −	− −	+ +
No treatment; primary check	Humid chamber	Red cellophane	− −	− −	+ +
	Humid chamber	Green cellophane	− −	− −	+ +
No treatment; secondary check	Open bench	Without cellophane	− −	− −	− −
	Open bench	Without cellophane	− −	− −	− −

* Positive responses in each of four pots indicated by + sign.

† Galls mostly discoid, efflorescent, about 1 cm. in diameter and 0.5 cm. in thickness. No significant differences in rate of development or size. Galls developed under red cellophane were pale yellow green; those developed under green cellophane were pale straw in color.

the attainment of the physical limits so indicated, a random series of plantlets developed on the galls. These appeared to originate from subsurface gall cells. The growth of the plantlets was ephemeral and no transplanting was carried out to determine the incidence of polyploidy.

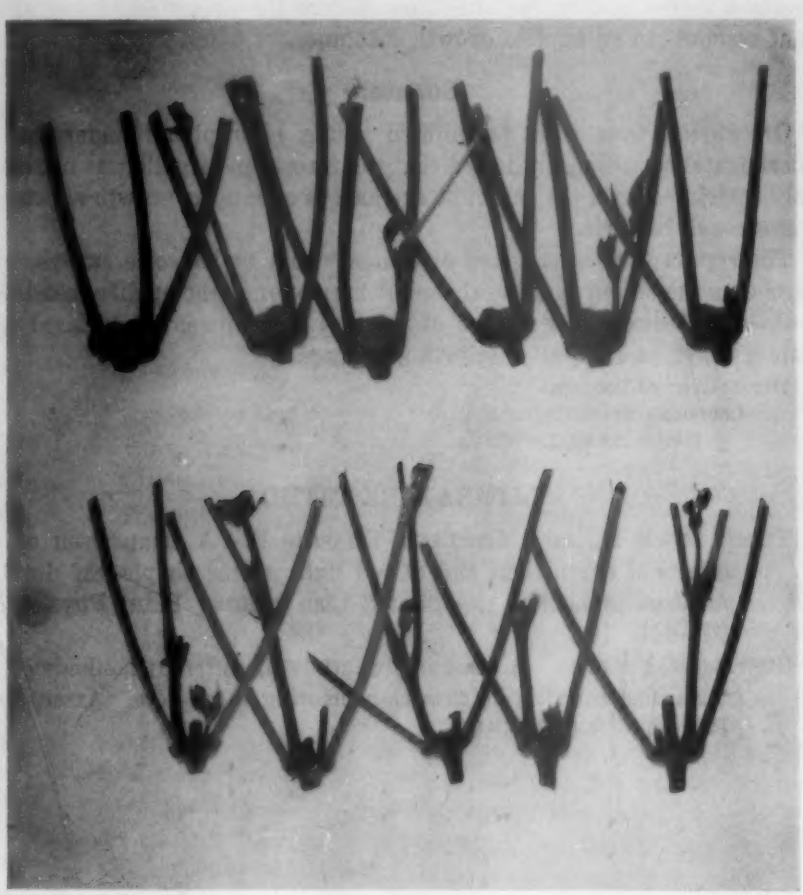

FIG. 1. Type of galls developed on decapitated young bean plants in the absence of any applied inciting agent. Above: plants from humid chambers. Below: plants from open greenhouse bench.

Discussion

The development of the symmetrical efflorescent type of gall shown in figure 1 was perhaps contingent upon the maintenance of abnormal environmental conditions about an abnormal or cut structure. Nevertheless there appeared to be an involvement of wound hormones which effectively obscured any plant reaction to indole-acetic acid or bacteria. Under the more normal environmental conditions of the open greenhouse bench no gall-formation took place, and the results obtained were in agreement with

those reported by GUSTAFSON (2) who obtained no gall-formation in control plants, even when cut ends were coated with lanolin.

There appeared to remain the interesting possibility, however, that under some conditions the potential production and effectiveness of internal wound hormones might modify, condition, or obscure the extent or nature of the plant response to an applied growth substance.

Summary

Greenhouse tests with decapitated young bean plants under various experimental conditions indicated that the formation of galls was not essentially correlated with the action of extraneous agents, as growth substances or crown-gall bacteria.

The results were interpreted as evidence that under some experimental treatments the action of natural wound hormones might modify, condition, or obscure the extent or nature of the gall development considered as a plant response to an applied growth substance.

DEPARTMENT OF BOTANY
LOUISIANA STATE UNIVERSITY
BATON ROUGE, LOUISIANA

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WOUNDING AND SUGAR TRANSLOCATION

F. W. Went and Marcella Carter

Translocation of auxin occurs across cuts without difficulty. On the other hand, translocation of sugars, virus, caulocaline (**1**), and the flowering hormone (**4**) is only possible through intact vascular tissue. Wounds near vascular tissue have an effect upon growth of remote regions (**2**). Therefore, the relation between wounding through removal of leaves and bleeding, or stem elongation, as tests for sugar translocation was investigated (**2**).

Tomato plants, when placed in darkness, lose their available carbohydrates within 30–50 hours. After this period all processes which require respiratory energy have dropped to a very low level. This level can be raised again 15–20 hours after application of a 10 per cent. sucrose solution to some of the leaves (**2**).

Effect of wounding on subsequent bleeding

When the above-ground parts of a tomato plant are cut off, the stump bleeds profusely for a few days. When plants have been kept in darkness previous to the cutting, bleeding is much reduced. By applying sucrose to one or more leaves during the period in darkness, bleeding is increased afterwards, indicating the applied sucrose has been translocated to the root system (**2**). Four groups of 60-cm.-tall San Jose Canner tomatoes were brought into a dark-room kept at 25° C. One group of 20 plants was left as controls and the other three groups had two leaves per plant submerged in 10 per cent. sucrose. In one of these groups all other leaves were removed with a sharp razor; in another group the leaves were pinched off between thumbnail and forefinger; and in the last group the plants were left intact. After 24 hours in darkness the tops of all plants were cut off, drip tubes were attached to the stumps, and the bleeding exudate collected. During the next four days the following amounts of exudate were collected (cc. per plant):

Intact plants, no leaves in sucrose	4.51 ± 0.48
Two upper leaves in sucrose, all other leaves cut off	7.86 ± 0.71
Two upper leaves in sucrose, all other leaves pinched off	9.00 ± 0.47
Two upper leaves in sucrose, no leaves cut	12.12 ± 0.95

Removing the leaves during sugar treatment greatly reduces the effectiveness of the applied sugar, more so when the leaves are cut than when they are pinched. It can be concluded from this and similar experiments that less than half the amount of sugar, transported in the intact plant, reaches the roots of the trimmed plant.

Effect of wounding on subsequent growth

Stem elongation in tomatoes stops 30–50 hours after placing them in darkness. Some time after leaves have been submerged in a 10 per cent. sucrose

solution, stem growth is resumed in darkness (3). This response to applied sucrose is quantitative, and can be used to measure the amount of sugar translocated from the supplying leaves to the growing zones of the stem.

Tomato plants (30–40 cm. tall) were placed in the darkroom and their stem length was measured daily. In all but one group two middle leaves were submerged in 10 per cent. sucrose, and then all leaves above or below them cut or pinched off. During the second day in darkness the stems grew in length as follows (mm.):

No leaves in sucrose, plant intact	3.8 ± 0.8
Two middle leaves in sucrose, leaves below and above cut	13.5 ± 1.6
Two middle leaves in sucrose, leaves above those in sugar cut	17.6 ± 1.5
Two middle leaves in sucrose, leaves below these in sugar cut	24.4 ± 2.0
Two middle leaves in sucrose, no leaves cut off	29.6 ± 2.3

In many similar experiments the effects of pinching and cutting off the leaves were studied. The following is a recent experiment with a typical response. Growth during first two days in darkness (mm.):

No leaves in sucrose, plant intact	6.2
Two lower leaves in sucrose, other cut off at insertion on stem	12.5
Two lower leaves in sucrose, others cut off 1 cm. away from stem	20.9
Two lower leaves in sucrose, others pinched off 1 cm. away from stem	22.6
Two lower leaves in sucrose, no leaves cut off	28.9

The effect of wounding wears off with time, as the following experiment shows. Four leaves were cut off several groups of tomato plants on 4 successive days, and after the leaves of the last group were cut off, all plants were placed in the darkroom and one leaf of each plant was submerged in 10 per cent. sucrose. Their growth (in mm.) was followed for the next 72 hours in darkness, with the following results:

No leaves cut off, one leaf in sucrose	21.6
Four leaves cut Nov. 21, one leaf in sucrose	12.6
Four leaves cut Nov. 22, one leaf in sucrose	11.2
Four leaves cut Nov. 23, one leaf in sucrose	4.3
Four leaves cut Nov. 24, one leaf in sucrose	7.2
No leaves cut off, no leaf in sucrose	11.2

These experiments can be interpreted along two main lines: either cutting releases substances inhibitory for both bleeding and stem elongation; or it interferes with sugar translocation. The first alternative is not likely. In the first place we do not know about inhibitory substances inside the plant, which are translocated over such distances; secondly, one would expect the release of more of those substances after pinching than after cutting with a sharp razor blade; and thirdly, it would not be expected that the length of petiole stump should have much influence on the inhibitory substances released. The explanation of interference with the sugar translocation in the stem is more direct; it satisfactorily explains the big effect of length of

petiole stump upon growth, and it can easily be brought in line with the cutting versus pinching effect.

Actual sugar determinations were made in tops of tomato plants kept in darkness and fed with 10 per cent. sucrose through one or two leaves. Some groups (of 7 plants each) were left intact, in other groups all of the leaves except those in sucrose were cut or pinched off. Results are given in table I.

In all except one case wounding decreased the amount of sucrose found in the tops, corroborating the conclusions reached in the preceding experiments.

Regardless of the theory of sugar translocation to which one subscribes, it will be agreed that inside the sieve tubes a high turgor pressure exists. Upon cutting, this pressure is released and it will tend to equalize in neighboring sieve-tube elements. This probably causes the well-known clogging

TABLE I

CONCENTRATION OF SUCROSE IN TOPS OF TOMATO PLANTS KEPT IN DARKNESS AND FED WITH 10 PER CENT. SUCROSE THROUGH ONE OR TWO LEAVES. RESULTS GIVEN IN PERCENTAGE OF DRY WEIGHT

EXPERIMENT	NO SUCROSE	ONE LEAF IN 10 PER CENT. SUCROSE	TWO LEAVES IN 10 PER CENT. SUCROSE
	%	%	%
Plant intact	0.00	0.14	0.55
Leaves pinched off 2 cm. from stem	.....	.....	0.36
Leaves cut off 2 cm. from stem	.....	0.62	0.41
Leaves cut off at insertion	.....	0.09	0.36

of sieve plates, which in most microscopic preparations are found to be covered with cell contents at the same side. Pinching presumably opens fewer sieve tubes, with consequently less clogging. Since the clogging effect must decrease with distance, petioles cut off some distance from the stem will cause less clogging of the stem sieve tubes, than when cut close to the stem. This clogging effect seems to decrease with time.

Experiments such as these may furnish further evidence as to the nature of sugar translocation in stems. They also may tend to explain controversies in cutting technique; in some cases sharp knives may be less desirable than cutting instruments of the crushing type.

Summary

Cutting off leaves close to the path of food movement seems to inhibit sugar translocation in stems. Pinching off the leaves seems to interfere less with the sugar translocation than cutting them off with a sharp knife.

WILLIAM G. KERCKHOFF LABORATORIES
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

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BRIEF PAPERS

A NOTE ON THE PRESENCE OF PYRUVIC ACID IN EBENEZER ONIONS[1]

EMMETT BENNETT

Pyruvic acid is one of the key substances formed during carbohydrate metabolism. If optimum conditions exist in the animal body, the acid is quickly converted into other compounds. Under anaerobic conditions, carboxylase produces acetaldehyde and carbon dioxide; by oxidative decarboxylation, pyruvic dehydrogenase produces acetic acid and carbon dioxide. It has been shown that carboxylase is a system composed of a protein, a divalent cation (Mn or Mg), and diphosphothiamine (prosthetic group) (**5**). Therefore, if there is a deficiency of thiamine, pyruvic acid accumulates and symptoms of the avitaminosis appear (**9**). Thiamine therefore is accorded the important rôle in carbohydrate metabolism. Little is known concerning the accumulation of pyruvic acid in the plant. The following report deals with the isolation of a compound believed to be pyruvic acid from Ebenezer onions, and with certain reactions with which this compound may be associated.

Experimental

In preparing to block the carboxylase activity of the onion to allow the accumulation of pyruvic acid, it was noted that a positive indicative test could be obtained without such treatment. Furthermore, when precautions were taken to inactivate the enzymatic system by heat treatment prior to mincing the tissue in a Waring blendor, a negative test was obtained; without heat treatment prior to mincing, the test was positive and the reaction was not affected by subsequent heating.

Ebenezer onions which had been in storage for about 3 months were used. The roots and dry skin were removed and the onions sliced and placed in a Waring blendor along with enough distilled water to carry the pulp. After blending for three minutes the suspension was filtered through cheesecloth and the filtrate subsequently treated with trichloroacetic acid. The filtrate, when treated with sodium nitroprusside, and then made alkaline with either aqueous or alcoholic sodium hydroxide, developed a deep red color. This color could be discharged by acid and restored by alkali. To determine the stability of the color-developing compounds, aliquots were treated with an acid and a base to yield a pH of approximately 1 and 11, respectively, and boiled for a few minutes. Positive tests were obtained in both cases. Distillation of aliquots of the original filtrate yielded distillates which gave negative tests. The isolation of the unknown compound and the production of a 2,4-dinitrophenylhydrazone were conducted substantially as outlined by PETERS and THOMPSON (**8**). The hydrazone when treated with alcoholic sodium hydroxide developed a deep red color which was quite stable in comparison with the color developed by the original substance in the initial stage. The crystals of the hydrazone, when recrystallized from hot ethanol,

[1] Contribution no. 523 of the Massachusetts Agricultural Experiment Station.

were greenish yellow diamond-shaped plates; the poles of the longer axis were pointed, while those of the shorter axis were curved or rounded (7). They melted at 213°–214° C. and contained 20.20 per cent. nitrogen.

For comparative purposes an aqueous solution of pyruvic acid of known origin was used which was prepared in the following manner. A commercial sample was subjected to two distillations at 65° C. and 15 mm. pressure. The final distillate was diluted to form a solution approximately 0.2 molar. Aliquots of this solution were used in the preparation of derivatives.

The crystals of the 2,4-dinitrophenylhydrazone of the pyruvic acid prepared above appeared to be identical in color and shape with those of the unknown, melted at 214° C., and contained 20.47 per cent. nitrogen (2). The carbon and hydrogen content of each of the two hydrazones differed by about 0.6 per cent.

In order to obtain some knowledge of the nature of the change indicated in the first paragraph, bisulphite-binding compounds were determined on distillates obtained from representative subsamples heated before and after blending. The results indicated that heating prior to blending caused a loss of about two-thirds of the bisulphite-binding substances.

Discussion

It should be pointed out that the red color developed by alkali hydroxides and sodium nitroprusside in the presence of such carbonyl compounds should not be considered conclusive. The nitroprusside test with concentrated ammonium hydroxide is reported to yield a violet blue color which is believed to be specific for pyruvic acid (2, 6). All substances which develop a red color in the presence of alkali hydroxides and nitroprusside, however, do not form 2,4-dinitrophenylhydrazones which will give a red color with the above reagents.

The treatment of biological carbonyl compounds with dilute alkali at 100° C. eliminates to a considerable extent alkali-labile compounds such as methyl glyoxal, acetaldehyde, glyceraldehyde, and dihydroxyacetone which may give a nitroprusside test with alkali hydroxides (3). Furthermore, the distillation of solutions containing such compounds serves to give an index of the kind of substance which may be present. For instance, methyl glyoxal is found in the distillate, whereas pyruvic acid is found in the residue. The isolation of the unknown compound by the method of PETERS and THOMPSON (8) or CASE (1) further eliminates compounds which do not contain both a carboxyl group and a ketone or aldehyde group. The presence of acids (other than those taking part in intermediary metabolism) which are capable of these reactions is exceedingly improbable. The latter have been sufficiently well studied to permit their identification (2, 4, 7).

The above data indicate that pyruvic acid may accumulate in the Ebenezer onion; and that certain bisulphite-binding compounds may be associated with the disappearance of pyruvic acid.

MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION
AMHERST, MASSACHUSETTS

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NOTES

W. W. Garner.—The American Society of Plant Physiologists conveys its felicitations to DR. GARNER on the occasion of his seventieth anniversary. As a tribute to his distinguished career as a scientist, the society presents a brief biographical record of DR. GARNER and his professional work.

DR. WIGHTMAN WELLS GARNER was born July 15, 1875, at Timmonsville, South Carolina. In 1896, he received the A.B. degree from the University of South Carolina, and in 1900, his Ph.D. from Johns Hopkins University. In 1904, he became Assistant Chemist in the Bureau of Chemistry of the United States Department of Agriculture. In 1905, DR. GARNER was transferred from the Bureau of Chemistry to the new Bureau of Plant Industry where he was assigned to assist MR. A. D. SHAMEL who was then conducting breeding experiments with tobacco and other crops. In the course of this work there arose the difficult problem of determining accurately the comparative burning qualities of the different selections of tobaccos. DR. GARNER undertook this problem and devised an apparatus in 1906 which showed the rate of burn of the finished cigar. He also originated an apparatus for testing the burn of tobacco leaves. Both devices were very useful in the scientific evaluation of tobacco samples.

In 1908, DR. GARNER was made Physiologist in Charge of Tobacco and Plant Nutrition Investigations and under his leadership the scientific study of all phases of tobacco culture in the United States was greatly intensified. He began fundamental studies of curing methods in the Connecticut area where shade types were extensively grown, chiefly for cigar-wrapper purposes. This problem had varied aspects due to the fact that each of the highly specialized types of tobacco is used for a different purpose. Further work was also begun under carefully organized supervision in cooperation with state officials in Maryland, Kentucky, Virginia, West Virginia, Pennsylvania, North Carolina, Florida, South Carolina, Tennessee, Georgia, and Wisconsin. As a result of this new and vigorous approach of forty years ago to the diverse problems of culture and curing of tobacco, there exists today a chain of efficient field stations in the chief tobacco districts, all of which have made significant contributions of the science of this important crop.

Production of various cover crops such as vetch, cowpeas, and others was also undertaken on tobacco soils to determine their value in rotation systems. In the field of pathology, studies were made of black root-rot, Thielaviopsis, mosaic disease of tobacco, and new methods of sterilization of seed beds were introduced. Important pioneer work on mineral nutrition was that connected with magnesium and the micro-elements, including boron,

in relation to the tobacco plant, the findings of which are applicable to many other crops. Studies of the nitrogen and potassium nutrition of tobacco, research on the occurrence of the alkaloid, nicotine, in the tobacco plant and its relation to the various factors of plant nutrition have all yielded outstanding results. A simple and accurate method for the determination of nicotine in tobacco was an outgrowth of this research. Cultural operations in the field have also been undertaken with success.

Studies of the flowering behavior of the Maryland mammoth strain of tobacco led to the discovery that length of day is a powerful influence in regulating the flowering, fruiting, and vegetative expressions of plants. The early papers of GARNER and ALLARD in this field initiated a new chapter in plant physiology. This discovery was not only a notable contribution to theoretical botanical science, but it also proved to be of great practical value in the control of flowering and fruiting of crop plants and the distribution of these in regions whose seasonal length of day is most favorable to production. There has perhaps been no recent botanical discovery which has had more far reaching effects or has so greatly added to the understanding of plant behavior in all parts of the world.

Under DR. GARNER'S direction, the Division of Tobacco and Plant Nutrition has investigated all phases of tobacco culture, including the genetics, mineral nutrition, and diseases of this crop as well as studies of the growth and nutrition of many other plants in weed, bare fallow, and crop rotations. DR. GARNER has furnished inspiration and guidance to a staff of capable scientific associates who have successfully completed much important research along many and varied lines. In addition to his administrative activities, he has published numerous individual and joint papers on various phases of tobacco chemistry and culture. Thirty-nine papers have been published in these fields including one on the oil content of seeds as affected by the nutrition of the plant. Twenty additional publications have dealt with photoperiodism.

DR. GARNER retired from the United States Department of Agriculture August 1, 1945, having devoted 41 years of his life to studies of the physiology of the tobacco plant from the field to the curing barn. As a result of his efforts a strong and loyal scientific organization was built up. The splendid record of scientific achievement of this organization speaks eloquently for itself. Outstanding results have been achieved in studies of the mineral and nitrogen nutrition of the tobacco plant, in improved knowledge of tobacco mosaic and other tobacco diseases, in data on nicotine relationships, and in studies of photoperiodism. The distinguished contributions in pure and applied science of this relatively small scientific unit in the United States Department of Agriculture under DR. GARNER'S administration have

won the world-wide esteem of scientists and agriculturists. As a result of his personal research, DR. GARNER himself has achieved distinction as a national and international authority in his field. In 1937, the honorary degree of Doctor of Science was conferred on DR. GARNER by both Clemson College and North Carolina State College in recognition of his outstanding record of public and professional service. In 1930, the Stephen Hales Prize for distinction in research was awarded to him by the American Society of Plant Physiologists.

Election of Officers.—The official results of the recent election of officers of the American Society of Plant Physiologists are as follows: President, PAUL J. KRAMER, Duke University; Vice-President, EARL S. JOHNSTON, Smithsonian Institution; Secretary, R. B. WITHROW, Purdue University; Member of the Executive Committee, D. R. HOAGLAND, University of California; Member of the Editorial Board of Plant Physiology, H. A. SPOEHR, Carnegie Institution.

Membership List.—New members, new subscribers and others who have not received a copy of the A.S.P.P. directory of members (Bulletin 15, 1944) may obtain the same from the secretary or the executive secretary-treasurer upon request.

Advances in Enzymology.—Volume V for 1945 of Advances in Enzymology and related subjects of biochemistry is a 268-page publication containing eight important papers by widely known specialists. In addition to very complete author and subject indexes to volume V, this number also contains cumulative subject and author indexes to volumes I, II, III, IV and V. The contents comprise: Physical and Chemical Properties of Tomato Bushy Stunt and Tobacco Mosaic Virus by N. W. PIRIE; Coagulation of Blood by ERWIN CHARGOFF; Amino Acid Decarboxylases by H. BLASCHKO; Alcoholic Fermentation of Oligosaccharides by J. LEIBOWITZ and S. HESTRIN; Pyruvate Metabolism by ELMER STOTZ; Biochemistry of Fusaria by F. F. NORD and R. P. MULL; Enzymatic Reactions of Nicotinamide and Related Compounds by F. SCHLENK; and Enzyme Reactions on Sulphur Compounds by C. V. SMYTHE. The volume is priced at $5.50 by the Interscience Publishers, 215 Fourth Avenue, New York City 3, N. Y.